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ORIGINAL ARTICLES

TREATMENT OF STRANGLES WITH SULPHANILAMIDE

By F. C. Minett, Imperial Veterinary Research Institute, Mukteswar, and Major W. P. S. Edwards, Indian Military Veterinary Laboratory, Lahore

(Received for publication on 24 July 1944)

(With 39 text-figures)

As part of a plan for the investigation of strangles in the Army Remount Depots of Mona and Sargodha in the Punjab, it was decided to make a systematic trial of sulphanilamide, leaving an equal number of controls treated by ordinary methods. The present paper gives the results so far obtained and the opportunity is taken to discuss the whole problem with an eye to the future. Strangles in young horses at these depots has always been a major problem and two papers dealing with the matter from the epizootiological standpoint have been published [Minett, 1944.]

Literature

The history and nature of the sulphonamide compounds, their dosage and views on their mode of action are matters which have been discussed by a number of authors and extensive repetition is unnecessary.

Some of the most recent information on the causal agent of strangles is furnished by Bazeley and Battle [1940] in Australia. From 415 cases of serious and typical equine diseases they isolated 457 strains of hæmolytic streptococci. All of these belonged to Lancefield's serological group C, in fact the horse is the commonest source of these organisms just as man is the usual source of group A streptococci and the cow of group B streptococci. By agglutination, five main types could be distinguished among the 457 strains while biochemically they fell into five types : (a) *Str. equi*, which fermented neither lactose, sorbitol nor trehalose; (b) 'equine 1' which fermented sorbitol; (c) 'equine 2' which fermented trehalose. Types (b) and (c) might or might not ferment lactose in addition and they are generally referred to as *Str. pyogenes equi*. All the strains isolated from fresh strangles pus were *Str. equi* (agglutinative type 1) and this type was also associated with some cases of respiratory catarrh.

There appears to be no doubt as to the sensitivity of group C streptococci to sulphonamide preparations. Stableforth [1938] reported that sulphanilamide would protect mice against 100 to 1000 or even more minimal lethal doses of culture. Descazeaux, Courtade and Rocq [1939] in similar tests used a virulent hæmolytic streptococcus isolated from a strangles abscess. An 18-hour serum broth culture of this organism in dose of 0·5 c.c. of 10^{-7} dilution intraperitoneally was regularly fatal in mice. In the tests 0·5 c.c. 10^{-4} dilution (i.e. 1000 fatal doses) was used and this killed nine out of the ten control mice within 48 hours and the remaining one within 72 hours. Of 10 mice similarly inoculated but also given sulphanilamide—at a dose of 0·01 gm. twice daily for two days, the first dose being given 30 minutes before the streptococci—four survived four or five days, three for six days and three for more than eight days.

As to the mode of action of sulphonamide drugs, there seems no longer any

doubt that they have a direct effect on bacterial metabolism (inhibition of an essential enzyme reaction) leading to bacteriostasis and that the phagocytic cells of the body are the actual killing agents. This effect was nicely shown by Gay and Clark [1937] in the case of an experimental streptococcus empyema in rabbits. The phagocytic cells themselves are unharmed by concentrations of the drug which are active against bacteria. McIntosh and Whitby [1939] have shown that the drugs are not simple germicides, although, as Colebrook, Buttle and O'Meara [1936] had pointed out for sulphanilamide, the drug is bactericidal on small numbers of hæmolytic streptococci in culture medium and in blood. It is also known that they are not immediately active *in vitro* or *in vivo*; they are active on highly virulent organisms and those in the logarithmic phase of growth, and there is a quantitative relationship between effective dose and the number of bacteria attacked. Further, it is decided that the drugs do not neutralize toxins, they do not stimulate the activity of leucocytes nor influence the production or quality of specific immune bodies. The compounds are easily diffusible in the body and are very effective in a medium which is a poor one for the organism, e. g. urine or blood. On the other hand, as Fleming [1941] has shown, pus inhibits the action of sulphanilamide, so that it should not be used on grossly septic wounds.

The sulphonamide drugs which are chiefly in use at the present time are sulphanilamide, sulphapyridine (M & B 693) and sulphathiazole. Sulphanilamide is capable of dealing with most hæmolytic streptococcus infections, is active against *Cl. welchii*, meningococci, gonococci, *Bact. coli* and *Proteus*, but is relatively inactive against staphylococci and pneumococci. Sulphapyridine is active against hæmolytic streptococci, pneumococci and *Cl. welchii* and has some action against staphylococci and *Cl. septicum*. Sulphathiazole is not only active against hæmolytic streptococci but is also definitely better than the other two against staphylococcus infections. Finally, it is to be noted that among bacteria belonging to susceptible species drug-resistant strains are sometimes met with. An apposite illustration of this was recently reported by Hendry [1942]. A horse undergoing immunization with group A hæmolytic streptococci developed a streptococcus septicæmia and died in spite of 10 days treatment with sulphanilamide. The streptococci isolated from the blood of the animal, the day before its death were considerably more resistant to sulphanilamide by *in vitro* tests than were the organisms used for immunization.

Since the early days when prontosil was found to be efficient in reducing the mortality in human puerperal fever, it has become apparent that the sulphonamides are of value in a number of other human infections, streptococcal and otherwise, particularly the more acute class of disease. However, their efficacy has not been proved in all cases, e.g. French [1939] in a series of painstaking and controlled observations was unable to demonstrate any useful action in scarlet fever.

All careful practitioners realize that the drug should be given as early as possible, in full dosage, at proper intervals, and for a sufficient length of time, say up to 10 days. Continuation without an interval beyond this time may lead to leucopenia and anæmia. If relapses appear, dosing should be recommended. Water intake should be restricted and medicines containing sulphur withheld. It is good practice if possible to make periodic estimations of the sulphonamide concentration in the blood, since the rate of absorption and excretion varies somewhat in different individuals. Bazeley, Jokobowicz and Splatt [1940] give a convenient dosage table for horses. Thus, to attain a concentration of sulphanilamide of 5 mg. per cent in the blood, a horse weighing 1000 lb. would require 90 gm. the first day and 60 gm. on subsequent days. For a 750 lb. horse the amounts

are 67 and 45 gm. During the present war, sulphanilamide, applied daily as a dusting powder, has been found useful in ridding wounds of hæmolytic streptococci [Colebrook, 1941]. In this case the wound should either be a fresh one or one in which granulation is proceeding. In fact, sulphanilamide can sometimes be used in this way when dosage by the mouth is contraindicated.

In some acute streptococcus infections in horses, e.g. pneumonia, sulphanilamide is of outstanding value [Descazeaux *et al*, 1939; Hignett, 1940; Steck, 1940; Bazeley, 1940-1]; in chronic conditions, e.g. chronic sinusitis or nasal catarrh, fistulous withers [Arnold, 1939; Bazeley, 1940] the drug is of less value. The following workers have reported on trials with sulphanilamide or related compounds in strangles (the numbers in parenthesis show the number of cases treated):

1938, Guerret (11).
1939, Douglas and Walker (3)
Descazeaux *et al.* (8)
Arnold (5)
1940, Steck (at least 32)
Knowles (15)
Bazeley (1)
1941, Jastrzebski (?)
Leo (31).
Kurzenhauser (?)

The salient points of this work are,

(1) Impressions as to the value of the treatment are in general somewhat variable. This is not surprising because the disease is not a killing one and the course and clinical manifestations are themselves rather inconstant. Also, cases acting as controls were not always kept.

(2) There is fair unanimity as to the favourable effect of the treatment on the fever. This in itself must mean that the drug has some influence on the infection.

(3) There are indications that the drug inhibits inflammatory œdema and so tends to localize abscesses. This is in conformity with medical experience, e. g. in the early work on puerperal fever it was noted that prontosil prevented the spread of infection to the parametrial tissues [Colebrook and Kenny, 1936]

(4) The drug has no effect on an abscess once it has formed. This again would be expected.

Particular reference may be made to Steck's work. He administered 80-110 gm. doses to adult horses, the drug being suspended in 3-4 pints of tepid water and given through a tube by the nasal route. If the case was responding, the temperature might be expected to have fallen by the second day. For example, on the second day after a single-dose treatment of 80 gm. the main daily temperature had fallen by 0·5° C. (0·9° F.) or more in 25 cases, by less then 0·5° C. in 13 cases, and had remained the same or risen in 10 cases. Single doses, however, usually gave only a transient improvement, while with continued dosage one had to guard against anæmia. Of 25 cases treated with repeated doses (up to seven) at one—or two-day intervals, 13 showed enduring improvement, in six the effect was feeble or not enduring and in six the course of the disease appeared to the uninfluenced. Two important rules to be observed were (a) strict rest during the fever, (b) rest during a convalescent period of three complete days without any fever and then gradual return to exercise.

PROGRAMME OF INVESTIGATION

The experiments were carried out during the period, October 1939 to May 1942, on the numbers of animals shown below:

	1939-40	1940-41	1941-42
Mona	23 (ordinary dose)	..	5 (ordinary dose) 7 (larger dose)
Sargodha	..	10 (ordinary dose) 3 (larger dose)	..
	23	13	12 Total 48 experimental cases.

One control animal was observed at the same time as the experimental case, except that for the three 'larger dose' horses treated at Sargodha 1940-41 only two suitable controls were available.

By 'ordinary dose' is meant 1 gm. sulphanilamide per 10 lb. body weight* daily for 10 consecutive days, while the 'larger dose' was intended to mean four doses each of 80 gm. on alternate days. Had the 'larger' dose programme been adhered to strictly, it would have meant giving the animals about half the total amount of sulphanilamide administered to those on 'ordinary' doses. Actually, a few got more and a few less than the intended dosage (see Results). Dosage was commenced on the day of admission to hospital or on the next day. The daily 'ordinary' dose was divided in half, each half being given morning and evening. The 0·5 gm. tablets supplied were powdered and suspended in a pint of water and given by the nasal route by means of a stomach tube [Bazeley, 1940]. The 'larger' dose was given in the morning and usually at a single administration. No other treatment was given to the experimental cases but in all cases if abscesses developed they were opened at the proper time. The controls were chosen at the same time if possible as the experimental cases and in making the selection discrimination was avoided, save that if anything the experimental one was the more severe. Body temperatures were recorded twice daily, morning and evening. Body weights were taken on admission and (usually) on discharge, records were kept of symptoms and general condition, and the duration of hospitalization was noted.

TABLE 1

Mona. Sulphanilamide treatment with ordinary dose

Serial No.	Number of animal	Age (months)	Body weight (pounds) On admission	Body weight (pounds) On discharge	Date admitted to hospital	Days in depot prior to strangles	Duration of stay in hospital (days)	Bodily condition
				Treated				
1	8067	18	640	..	27—1—40	23	25	Condition good, died
2	8066	19	680	..	10—3—40	65	34	Lost condition, fair
3	8069	17	800	..	12—3—40	67	32	Good
4	8175	11	660	..	13—3—40	68	58	Severe attack, condition fair, good
5	50	8	430	..	13—3—40	103	31	Condition good, slow in picking up
6	52	8	480	..	18—3—40	103	26	Good
7	8226	12	680	..	18—3—40	12	42	Good
8	8181	12	660	..	19—3—40	17	73	Severe attack, lost condition during treatment, improved later
9	8188	13	650	..	19—3—40	16	25	Good
10	8215	16	740	..	19—3—40	13	75	Severe attack, lost condition during treatment, improved later
11	8251	12	650	..	20—3—40	13	51	Lost some condition and was slow in picking up
12	8195	18	730	..	20—3—40	17	42	Ditto
13	8190	11	560	..	20—3—40	17	29	Good
14	44	10	580	..	21—3—40	111	24	Good
15	8194	11	640	..	22—3—40	19	38	Good
16	8168	12	670	..	1—4—40	30	39	Good
17	8148	12	570	..	1—4—40	29	34	Lost some condition and was slow in picking up
18	8242	14	650	..	4—4—40	28	40	Good

*This is equivalent to rather more than 3 drachms per cwt. body weight.

Serial No.	Number of animal	Age (months)	Body weight (Pounds) On admission	Body weight (Pounds) On discharge	Date admitted to hospital	Days in depot prior to strangles	Duration of stay in hospital (days)	Bodily condition
					Treated (Contd.)			
19	8081	19	680	..	10—4—40	38	21	Good
20	8244	13	620	..	10—4—40	34	30	Good
21	8131	19	670	..	13—4—40	58	39	Lost some condition and was slow in picking up
22	8147	13	700	..	13—4—40	41	27	Good
23	8217	16	690	..	17—4—40	42	20	Good
24	181	16	812	840	24—3—42	334	38	Good
25	204	12	620	644	31—3—42	298	40	Good
26	202	17	728	756	2—4—42	300	29	Good
27	215	19	780	800	2—4—42	296	38	Good
28	252	14	616	654	7—5—42	36	30	Good
					Controls			
1	8070	12	650	..	Dates corresponding to above	26	30	Good
2	8068	18	780	..		65	38	Good
3	8077	16	730	..		38	32	Good
4	8192	12	580	..		10	24	Good
5	48	8	480	..		103	49	Temporarily lost some condition
6	8198	13	750	..		12	37	Severe attack, lost condition, improved later
7	8189	12	660	..		15	49	Ditto
8	8222	12	640	..		13	18	Good
9	8185	12	620	..		16	52	Severe attack, lost condition during attack, improved later
10	8246	12	640	..		12	52	Good
11	8213	13	660	..		14	29	Good
12	8187	14	580	..		17	40	Good
13	8225	15	700	..		14	32	Lost condition, died
14	8197	13	670	..		18	29	Good
15	51	8	336	..		19	26	Good
16	8171	13	660	..		29	28	Good
17	8180	12	690	..		29	30	Good
18	8256	14	670	..		8	36	Good
19	8151	17	750	..		38	11	Good
20	8220	13	700	..		35	48	Good

Table II

Sargodha. Sulphanilamide treatment with ordinary dose

Serial No.	Number of animal	Age (months)	Body weight (pounds)		Date admitted to hospital	Days in depot prior to strangles	Duration of stay in hospital (days)	Bodily condition
			On admission	On discharge				
				Controls (Contd.)				
21	8154	13	730	..	Dates corresponding to above	41	27	Good
22	8232	14	760	..		37	22	Good
23	8267	14	650	:		18	9	Good, died
24	128	16	700	700		36	38	Good, lost condition in hospital
25	208	12	620	588		21	40	Good, lost condition in hospital
26	206	18	756	784		23	29	Good
27	235	19	750	700		21	38	Good, lost condition in hospital
28	296	16	616	616		36	30	Good, lost and regained condition
				Treated				
29	5359	18	602	641	22—1—41	128	39	Fair
30	5327	14	621	645	25—1—41	131	39	Fair
31	5358	15	604	670	25—1—41	131	39	Fair
32	5341	15	505	556	22—1—41	134	35	Fair
33	5352	12	575	626	28—1—41	134	35	Fair
34	5975	14	500	571	14—2—41	76	35	Fair
35	6002	15	520	588	21—2—41	83	32	Fair
36	6087	10	506	570	21—2—41	63	43	Fair
37	6040	19	525	565	22—2—41	83	37	Fair
38	6045	16	545	591	22—2—41	84	42	Fair
				Controls				
29	5360	19	610	676	Dates corresponding to above	128	38	Good
30	5361	17	650	721		130	40	Fair
31	5335	18	625	715		131	39	Fair
32	5351	24	604	681		134	36	Fair
33	5325	14	607	792		134	36	Fair
34	6094	13	550	545		56	75	Rapid loss of condition while in hospital, improving very slowly
35	6001	16	522	600		81	39	Fair
36	5995	14	528	600		83	38	Fair
37	5344	14	538	640		158	68	Fair
38	5321	21	540	650		159	37	Fair

TABLE III

Sulphanilamide treatment with larger doses

Serial No.	Number of animal	Age (months)	Body weight (pounds) On admission	Body weight (pounds) On discharge	Date admitted to hospital	Days in depot prior to strangles	Duration of stay in hospital (days)	Bodily condition
				Mona (Treated)				
1*	32	15	650	700	24—12—41	31	38	Good
2†	33	14	700	740	26—12—41	33	36	Good
3	66	15	672	680	26—12—41	23	45	Good
4	27	17	710	710	25—1—42	64	24	Good
5	9964	15	786	820	6—1—42	82	25	Good
6	97	15	760	756	30—1—42	22	27	Good
7	103	16	750	784	31—1—42	23	33	Good
				Sargodha (Treated)				
8	6050	15	450	..	28—1—41	59	99	Poor, died
9	6077	12	470	520	27—1—41	38	103	Poor, improved later
10	5969	15	..	..	16—4—41	137	23	Ditto
				Mona (controls)				
1	74	14	775	790	Dates corresponding to above	20	38	Good
2	18	14	728	756		35	45	Good on admission, slight loss during first week
3	69	14	725	672		23	35	Good on admission
4	100	17	774	758		18	30	Good on admission
5	9986	15	662	700		68	25	Good
6	99	17	740	728		22	27	Transient loss of condition
7	105	15	734	756		23	33	Good
				Sargodha (Controls)				
8	6029	16	..	..	28—12—40	28	87	Poor, died
9	5954	16	..	..	29—3—41	..	56	Poor, died

*Was given four doses each of 70 gm.
†Was given three doses each of 75 gm.
Animals at Mona and Sargodha were received from Montgomery and Shahpur Districts (Punjab), respectively.

Results

These are summarized in Tables I, II and III, the results for Mona and Sargodha being shown separately. Temperature curves of many animals are also shown in Figs. 1 to 39. Additional notes on the cases are as follows:

Cases treated with ordinary doses

(Serial numbers given below correspond with the serial numbers given in Tables I, II and III. Numbers of cases in heavy type were treated with sulphanilamide, those printed in ordinary type are the corresponding controls).

Mona (Table I)

1. Died. P.m: three large mesenteric abscesses, of which one had burst.

2. Submax. and temporal abscess opened, 5 and 19 days. Purpura-like lesions developed during treatment. Legs swollen.

3. Submax. swelling subsided. Parotid abscess opened, 20 day.

4. Submax. abscess began to subside during treatment but formed again later and had to be opened, days 18, 28 and 30.

5. Parotid swelling which subsided.

6. Submax. abscess opened, 7 day.

7. Submax. abscess subsided during treatment. Temporal abscess opened later 25 day.

8. Submax. abscess subsided during treatment but this and parotid abscess formed later and had to be opened on five occasions between days 22 and 30.

9. Submax. abscess opened, 6 and 12 days.

10. Submax. abscess began to subside during treatment but later submax. and parotid abscesses and abscesses on cheek had to be opened, days 23, 24, 28, 30.

11. and **12.** Submax. swellings which failed to develop.

13. Submax. abscess opened, 17 day.

14. Submax. abscesses opened, days 4, 9, 12.

15. Submax. abscess subsided. Parotid abscess opened, 27 day.

16. Submax. abscess subsided during treatment but formed again later and had to be opened, 29 day.

17. Submax. abscess which failed to develop. On admission, parotid region tender and animal roaring.

18, 19 and **20.** Submax. abscess opened. In No. **20,** for three days there was some oedema of the legs and below abdomen.

21. Submax. abscess failed to develop. For four days, there was some oedema of the legs.

22. Submax. abscess opened, 10 and 16 days.

23. Submax. abscess opened, 10 day.

24. Catarrh. Submax. abscess opened, 10 day. Parotid swelling subsided. Mild case, convalescent from 13 day.

25. Submax. and parotid swellings subsided. Moderately severe case on admission, mild later. Convalescent from 10 day.

26. Catarrh. Submax. abscess opened, 9 day. Mild case, convalescent from 22 day.

27. Catarrh. Submax. swelling subsided. Mild case, convalescent from 20 day.

28. Submax. swelling opened, 7 day. Mild case, convalescent from 22 day.

1. Abscess in temporal region and of near shoulder opened, 6 day.
2. Parotid abscess which failed to develop.
3. 4. Submax. abscess opened, 6 and 5 days, respectively.
5. Submax. and parotid abscesses opened, days 4, 14, 17, 25. Slight roaring during first 16 days. For 6 days, oedema of hind legs.
6. Submax. and cheek abscesses opened, 8, 15 and 16 days.
7. Submax. and temporal abscesses opened on nine occasions between days 8 and 29.
8. Submax. abscess opened, 7 day.
9. Submax. and parotid abscesses opened, on five occasions between days 6 and 25.
10 and 11. Submax. abscesses opened, 13 and 6 days, respectively.
12. Submax. abscess failed to develop.

13. Submax. and temporal abscesses opened on days 17, 18, 19. Subsequently, parotid swellings developed. Died on 31 day. P.m: abscess over axillary lymph glands. Trachea full of 'thick, clotted foam'. No pneumonia.
14. Submax. abscess opened, days 4, 5, 7.
15. „ „ „ „ 6, 7, 9, 20.
16. „ „ „ „ 4, 13.
17. „ „ „ „ 5, 6, 19.
18. 19. Submax abscess opened, days 7 and 3, respectively.
20. Catarrh. Parotid abscess failed to develop.
21. Submax. abscess failed to develop.
22. Temporal abscess opened.
23. Died. P.m: post. mediastinal abscess and gangrenous pneumonia.
24. Catarrh. Submax. abscess opened, 14 and 18 days, parotid and temporal abscesses opened, 31 and 33 days, respectively. Fairly severe case with protracted course.
25. Catarrh. Off-feed. Submax. abscess opened, 12 and 13 days. Protracted case, convalescent from 24 day.
26. Catarrh. Submax. swelling opened, 4 and 10 days. Mild case, convalescent from 21 day.
27. Catarrh. Off-feed. Submax. and temporal abscesses opened, 7 and 24 days, respectively. Parotid swelling subsided. Protracted case, convalescent from 27 day.
28. Off-feed. Submax. abscess opened, 11 day. Parotid swelling subsided. Moderately severe case, convalescent from 22 day.

Sargodha (Table II)

29. Submax. and parotid abscesses opened, 5 and 21 days.
30. Submax. abscess opened, 9 day.
31. Submax. abscess opened, 26day. Parotid swelling subsided.
32. Submax. abscess opened, 13 day. Swelling over parotid with slight roaring.
33. Submax. abscess opened, 19 day.
34. and 35. Small submax. abscess formed after discharge from hospital.
36. Small submax. abscess opened, 31 day.
37. Submax. abscess opened, 36 day.
38. Submax. swelling subsided.

29 and 30. Submax abscesses opened, 13 and 4 days, respectively. Mild cases.
31. Submax. and parotid abscesses opened, 20 and 28 days. Severe case.
32. Submax. abscess opened, 6 day. Severe case.
33. Submax. abscess opened, 10 day. Severe case. Transient laminitis.
34. Submax. swelling subsided. Severe case.
35. 36. Submax. swelling subsided. Mild cases.
37. Heavy catarrh. Submax. abscess opened, 35 day. Moderately severe case.
38. Submax. abscess opened, 37 day. Mild case.

NOTE. Cases 29 to 33 and corresponding controls were all from one paddock of 25 animals all of which except 9 developed strangles.

Cases treated with 'larger' doses (Table III)

Mona

1. Sulphanilamide, four doses each of 70 gm. on 1, 3, 5 and 7 days, half the dose in morning and half in evening.
Submax. abscess opened, 7 and 18 days. Parotid swelling subsided. Moderately severe case at first, later mild. Convalescent from 22 day.
2. Sulphanilamide, three doses each of 75 gm. on 1, 3, and 5 days, half dose in morning and half in evening.
Catarrh. Submax. swelling opened, 10 day. Parotid swelling subsided. Mild case, convalescent from 17 day.
3. Sulphanilamide, 3 doses each of 80 gm. on 1, 3 and 5 days, whole dose at one time.
Catarrh. Submax. abscess opened, 17 day. Parotid swelling subsided ; roaring for three days. Severe case at first, later mild. Convalescent from 22 day.
4. Sulphanilamide, four doses each of 80 gm. on 1, 3, 5 and 7 days, whole dose at one time.
Submax. abscess opened, 4 day. Mild case, convalescent from 8 day.

5. Sulphanilamide as No. 4.
Catarrh. Submax. swelling subsided. Mild case, convalescent from 13 day.

6. Sulphanilamide, seven doses each of 80 gm. on 1, 3, 5, 7, 9, 11 and 13 days. Whole dose at one time.
Submax. abscess opened, 8 day. Convalescent from 18 day.

7. Sulphanilamide, 10 doses each of 80 gm. on 1, 3, 5, 7, 9, 11, 13, 15, 17 and 19 days, whole dose at one time.
Catarrh. Submax. abscess opened, 17 and 18 days. Parotid swelling. Maturation of abscess delayed, but convalescent from 19 day. Did not respond well to sulphanilamide.

1. Submax. abscess opened 7, 12, 13 days. Abscess on cheek opened, 13 day. Mild case, convalescent from 26 day.
2. Submax. and parotid abscesses opened, 9 and 15 days, respectively. Convalescent from 21 day.
3. Catarrh. Submax. and cheek abscesses opened, 10 and 13 days, respectively. Parotid abscess opened 19 and 21 days. Roaring from 3-10 days. A severe case with swelling of face and succession of abscesses. Convalescent from 24 day.
4. Submax. abscesses opened 8 and 12 days, cheek and temporal abscesses opened, 9 and 12 days, respectively. Convalescent from 19 day.
5. Only symptom was pyrexia, but 13 days after discharge from hospital readmitted with submax. abscess. Convalescent from 16 day.
6. Catarrh. Submax. abscesses opened, 7 and 11 days. Mild case, convalescent from 18 day.
7. Catarrh. Submax. abscesses opened, 6, 7 and 10 days. Mild case, convalescent from 14 day.

NOTE. For cases 1 to 2 and 1 to 7, animals were selected at time of admission to hospital. On each occasion two cases with pyrexia and submaxillary swelling, with or without other symptoms, were picked out. Usually at this time both were of about the same severity but, if any difference, the more severe one was taken for sulphanilamide treatment.

Sargodha

8. Sulphanilamide, three doses each of 82 gm. on 12 March, 15 March and 19 April 1941.
Strangles developed previously, on 28 January 1941. Condition failed to improve, fed little and had frequent colic. Condition thereafter rapidly deteriorated and was very poor when sulphanilamide was started on 12 March 1941. Condition improved for a time and animal took normal feeds, but condition again deteriorated and colic reappeared. Animal became very dull and weak and was off-feed. Died on 7 May 1941. P. m: large abscess in lung and two small abscesses on pleura.

9. Sulphanilamide, two doses each of 82 gm. on 4 April and 7 April 1941.
Strangles developed on 27 January 1941 but animal did not recover. Condition very poor and feeding little, when sulphanilamide treatment started on 4 April. Condition thereafter improved and body weight increased. Discharged cured.

10. Sulphanilamide, three doses each of 82 gm. on 17, 19 and 22 April 1941.
Fresh case. Heavy catarrh and feeding little. After treatment, condition improved. Discharged cured.

8. Had strangles on 12 April 1941 but did not recover. Condition poor but feeding well. Died on 4 April. P.m: large abscess in lungs ; kidney 'full of pus'.

9. Strangles developed on 29 March 1941. Condition declined and horse was off-feed. Submax. abscess opened on 13 April 1941. Appeared to be recovering and was feeding fairly well. Showed colic. Died on 24 May 1941. P.m: large abscess in the intestines.

Duration of hospitalization. The Veterinary Officer in charge at Mona (in June 1940) writes 'in most cases during administration of the drug abscesses showed signs of absorption and in five cases they absorbed completely; but in others they reappeared and had to be opened.

The period of treatment was thus lengthened. In some cases abscesses became multiple. Animals maintained their usual condition during treatment, only losing a little condition according to the severity of the attack and without relation to the administration period of the drug. After treatment, animals quickly regained condition except in those cases where abscesses were completely absorbed. These were slow in picking up condition. In the doses used, sulphanilamide......tends to suppress abscesses. The duration of treatment may be considerably shortened by maturing abscesses by normal methods and by using sulphanilamide after abscesses have been emptied. As used in the depot, the efficacy of the drug is most marked during convalescence. In larger doses, sulphanilamide may perhaps sterilize primary abscesses altogether'.

In a non-fatal disease such as strangles it might be thought that the length of stay in hospital would be an index of some value in deciding the efficacy of a treatment. Actually, for various reasons observations on this point are said to be notoriously unreliable.

In spite of the above remarks of the Veterinary Officer, examination of the figures shows that in the present series there was no difference in the period of hospitalization as between sulphanilamide-treated and control horses. Thus, from Tables I-III there were 43 treated cases and 42 untreated cases which recovered—excluding Nos. 8181 and 8215 of Table I and Nos. 6077 and 6094 of Table III, severe cases which were hospitalized for exceptionally long periods; the average time spent in hospital by the treated cases was 34·5 days and by the untreated 35·4 days.

Body temperature. Capt. Short, writing of the 23 cases treated at Mona in 1939-40, states: 'During the 10 days' course of treatment the temperature showed a tendency to come down in practically all cases and in some was normal after the first or second day and remained so throughout. In many cases it rose again after completion of the course'. Major Coulden, in regard to the cases treated at Mona 1941-42, states : 'The period of pyrexia was reduced by a few days'.

In Figs. 1-39 is reproduced the mean daily body temperature (mean of morning and evening readings) of treated animals and their controls from the day of admission to (in many cases) the day of discharge. It will be seen that in most cases the temperature falls within 7-10 days of admission without any sulphanilamide treatment, so that the similar fall which occurs in the sulphanilamide-treated animals clearly cannot be ascribed to the drug. In certain cases, however, (Nos. 52, 50, 8188, 8251, 181, 44, 9964, 27, 66, 8244, 8181) the early temperature fall in the treated animals is distinctly more abrupt than in the controls, so that here we may agree with the suggestion that the drug had some effect in depressing the temperature.

In their general outline, the paired temperature curves may be grouped as follows:—

(a) Cases where the control curve runs at a more or less constantly higher level than that of treated animals.
Nos. 52, 50, 8188, 8251, 181, 44, 8066, 27, 9964, 204, 97, 252, 33, 215 (Figs. 1 to 14). Total 14.

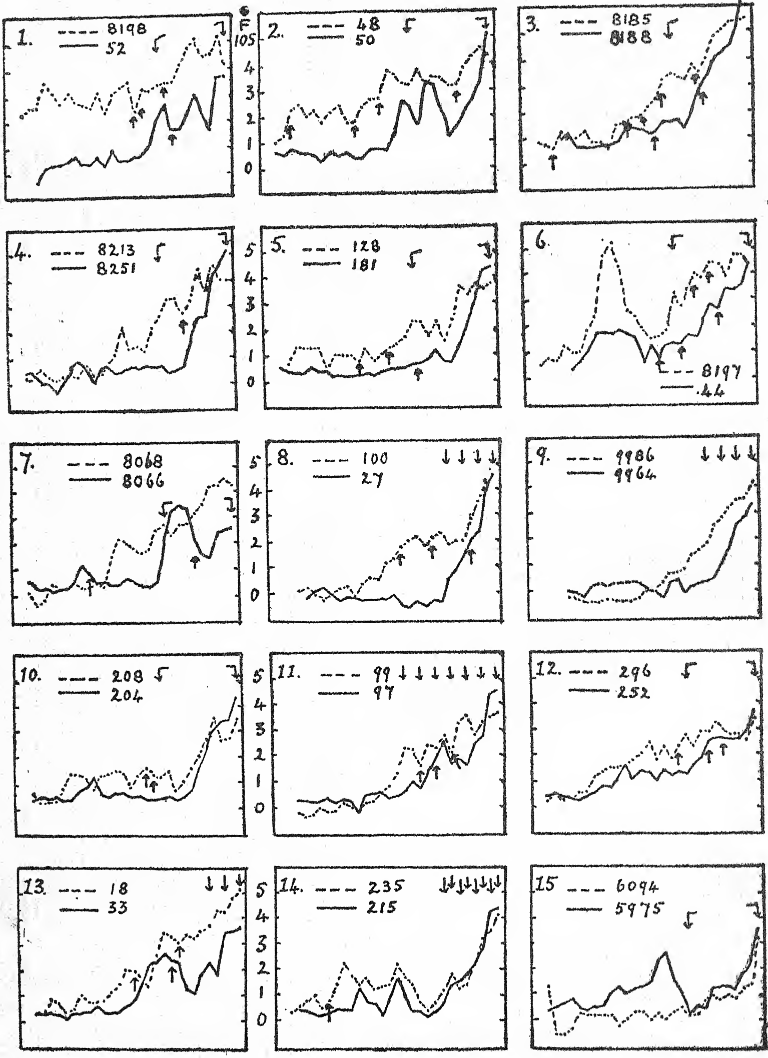
(b) Cases where the temperature curve of treated animals is at a higher level than the controls.
No. 5975 (Fig. 15). Total 1.

(c) Cases where on the whole there is little difference between the curves.
Nos. 5352, 66, 32, 103, 8131, 8195, 5327, 5341, 6040, 5359, 202, 6045, 6002, 6087 8194 (Figs 16 to 30). Total 15

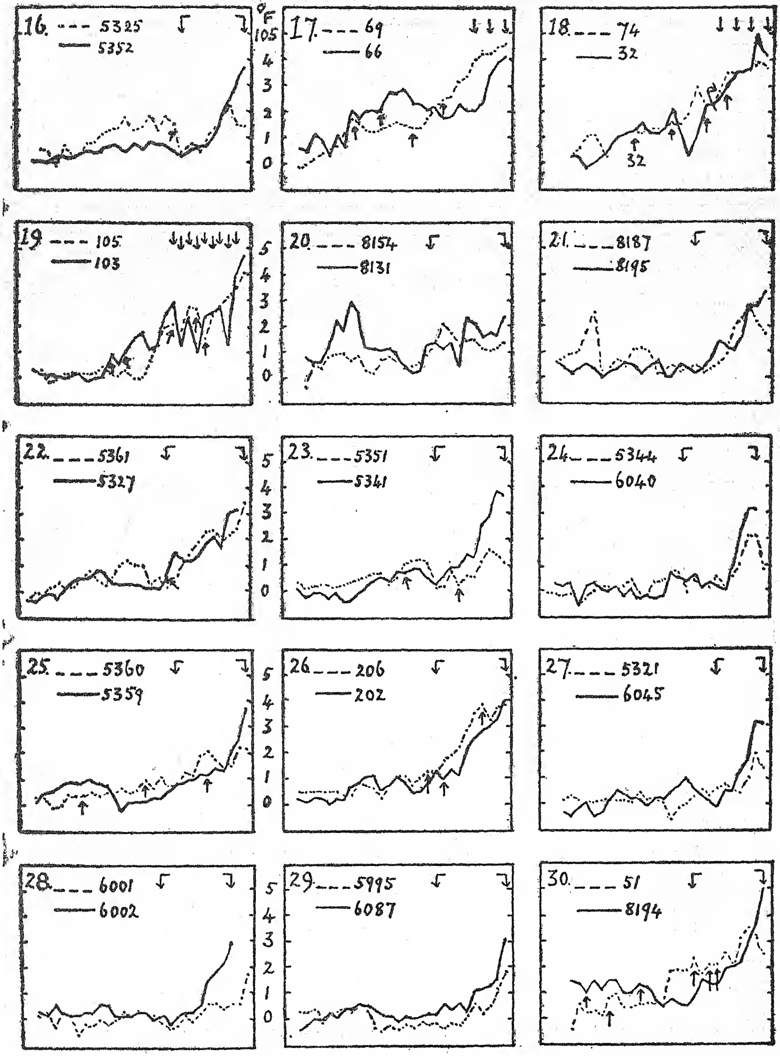
(d) Cases where a relapse during stay in hospital caused a second rise of temperature.

(i) in experimental cases
Nos. 8181, 8067, 8242, 8069, 8215, 8168 (Figs. 31 to 36). Total 6.

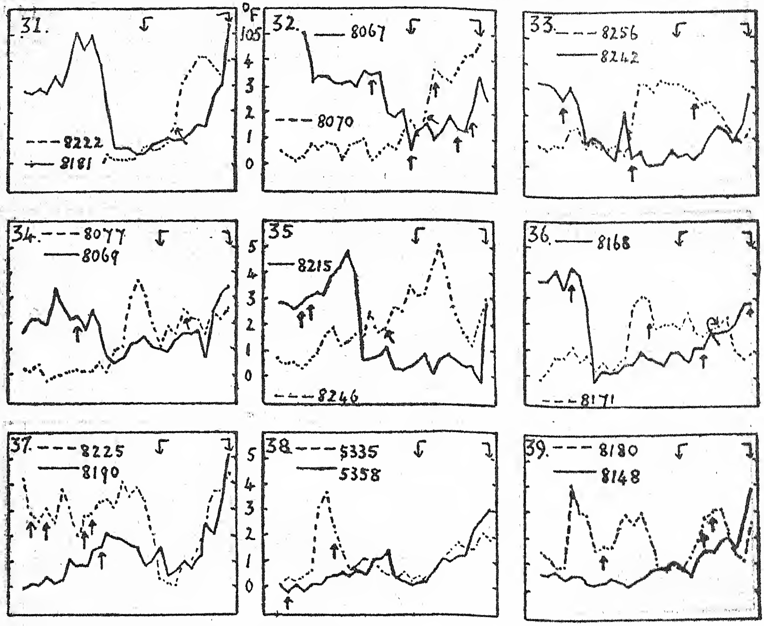
NOTE—In all these cases, the temperature of the control ran higher than the treated case during the period covered by the sulphanilamide treatment.



°F
105
4
3
2
1
0
5
1. ---- 8198
— 52
2. --- 48
— 50
3. --- 8185
— 8188
4. --- 8213
— 8251
5. --- 128
— 181
6. --- 8197
— 44
7. --- 8068
— 8066
8. --- 100
— 27
9. --- 9986
— 9964
10. --- 208
— 204
11. --- 99
— 97
12. --- 296
— 252
13. --- 18
— 33
14. --- 235
— 215
15 --- 6094
— 5975



°F
105
4
3
2
1
0
16. --- 5325
—— 5352
17. --- 69
—— 66
18. --- 74
—— 32
32
19. --- 105
—— 103
20. --- 8154
—— 8131
21. --- 8187
—— 8195
22. --- 5361
—— 5327
23. --- 5351
—— 5341
24. --- 5344
—— 6040
25. --- 5360
—— 5359
26. --- 206
—— 202
27. --- 5321
—— 6045
28. --- 6001
—— 6002
29. --- 5995
—— 6087
30. --- 51
—— 8194



—— S'amide-treated. ---- Untreated control.

↱ ↴ period of treatment. ↓↓ single doses.

↑ submax. abscess opened (control or treated case).

(ii) in control cases

Nos. 8190, 5358, 8148 (Figs. 37 to 39). Total 3.

NOTE. In these cases, the temperature of the control and its treated comrade were not dissimilar before the relapse.

NOTE. In all such case as (d), a resumption of sulphanilamide would be clearly indicated.

(e) Ungrouped cases.

Nos. 8081, 8217, 8175, 8244, 8226 Total 5.

Since it seems that for the purpose of assessing the influence of sulphanilamide on the temperature (a) and (d-i) can be combined, there are 20 cases—all of them at Mona—where the drug can be said to have had a favourable effect. In one case (b)—at Sargodha—the drug was without effect and in 15 cases (c)—eight of them at Sargodha—the drug may have had an influence on the body temperature, but if so this was not obvious on comparison with the control. The results of the larger series of cases at Mona are thus rather more promising than those at Sargodha.

Discussion

We think it must be concluded that sulphanilamide is of some definite value

in equine strangles. This would appear to be proved by the effect of the drug on the temperature curve, noted by other authors and also independently in the present series of cases by ourselves and the Veterinary Officers on the spot. There is also the evidence that the streptococcus of strangles is susceptible *in vitro* to the drug. It should be noted, however, that in strangles we are dealing with a streptococcus which has a pronounced tendency to cause suppurative changes; in the tissues, just the sort of morbid changes in which sulphanilamide is least likely to produce a dramatic effect. Granting, however, that the drug has some value, the question to be decided, perhaps, is how *at the present time* the sulphanilamide drugs should be used, since owing to the cost (prewar price of sulphanilamide in India was about 18 shillings per lb.) they could scarcely be given to every affected animal. Certainly in outbreaks of the magnitude commonly encountered in remount depots rigid selection of cases to be treated would be required, especially as half measures are likely to be of little value and an expenditure of at least 1 lb. of sulphanilamide is to be predicted for each adult horse. What is important is that sulphanilamide should not be used in practice in a routine or stereotyped manner. As Whitby [1939] has put it: 'Both clinical judgement and common sense are necessary to get the best effect with these drugs'. Admittedly, more information is required but in the circumstances it would seem best to reserve sulphanilamide (a) for young stock on stud farms, because they are valuable and the cost of treatment is relatively less; (b) for strangles cases which are not running the normal mild course; (c) probably most important for very early cases where there is a good chance of reducing fever, of inhibiting abscess formation and possibly of leaving behind a serviceable naturally acquired immunity.

In any event the policy may be adopted of giving larger doses during the first two days and then continuing with smaller doses—but not less than 1 gm. per 10 lb. body weight—up to 10 days. It may also be advantageous to divide the daily dose into three or four parts instead of into two parts.

In connection with (b) the widespread and careful use of the clinical thermometer should be urged. Officers should not leave the taking of temperatures in the hands of subordinates without first ascertaining that they are able to record accurately. It is particularly important that the bulb of the thermometer be introduced as far as possible into the rectum and be in contact with the mucosa. Temperature-recording should be continued for some little time after an initial attack, as a rise of temperature during this period may mean the onset of pyaemia. Unfortunately, we do not yet know just what influence the drug may exert on the pyaemic complications of strangles, but as such complications are the main source of mortality it would be right to test the drug on such cases. At the same time, the possibilities of sulphanilamide as a local dressing for abscess cavities, which are not closing with ordinary rapidity, might well be borne in mind. For this purpose the cavities should first be well opened up and thoroughly cleaned of pus.

The words 'at the present time' (above) have been emphasized because production costs may come down and because, as Fleming [1941] has pointed out, the field of therapeutics is expanding with the discovery of new sulphonamides. One may with some confidence anticipate the marketing of still more potent drugs, and also the elaboration of other very active antibacterial agents formed during the course of bacterial or mould growth. For instance, the product known as penicillin, which was obtained in 1929 by Fleming from a *Penicillium* mould, has a bacteriostatic effect on haemolytic streptococci 100 times as powerful as sulphanilamide and 20 times as powerful as sulphapyridine. Moreover, penicillin is not inhibited by pus. Penicillin has now been considerably purified,

and, as there are indications that the purified reagent is both stable and an intensely active bacteriostatic, its use in practice may well become more general. At the same time, success is being achieved in the preparation of other antibacterial products from cultures of various bacteria belonging to the *Bacillus* and *Actinomyces* groups.

As mentioned above, the sulphonamide drugs have a bacteriostatic effect and it remains for the body cells to complete the process of destroying the invading organisms. If the efficiency of the body cells in this respect can be augmented, the therapeutic results should be improved. Loewenthal [1939] has shown that in streptococcus infections in mice a combination of specific immune serum and sulphanilamide is far more effective than either reagent alone. Now that Bazeley [1940-2, 1942] has shown that a vaccine of some efficiency can be prepared from *Str.equi*, there is a possibility of increasing the resistance of horses exposed to strangles and the opportunities for chemotherapy may be correspondingly enhanced.

One last point that may again be emphasized is the importance of strict rest for strangles cases, both during the attack and during convalescence.

Summary

Sulphanilamide is of some value in the treatment of equine strangles. As would be expected from the nature of the disease, the results are not apparent in all cases. The beneficial effects consist mainly in a reduction of fever and probably to some extent in the localization of abscesses.

While the question of adequate dosage and duration of treatment is always important, sound clinical judgement is required in using the sulphonamides. Thus, in outbreaks of strangles, sulphanilamide might be used on animals in which the disease is not running the normally mild course. It would probably be still more profitable to use the drug on very early cases when fever is a pronounced symptom. There is need for further properly controlled enquiry on cases selected as falling within these two categories.

The possibility of combining sulphanilamide treatment with improved methods of vaccination is a matter for the future.

Acknowledgement

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PIROPLASMOSIS OF THE DOMESTIC FOWL IN NORTHERN INDIA

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(With Plate I)

The available literature shows that avian piroplasmosis has been only rarely and doubtfully recorded in India, the earliest reference to the subject being that by Lingard and Jennings [1904] who claimed to have seen pyroplasms in the blood of the British breeds of fowls in this country. Since these authors have not mentioned anything about the morphology of the parasite encountered by them, it is difficult to judge whether they were actually dealing with what is now known as *Aegyptianella pullorum* Carpano, or with some other haematozoon. The symptoms reported, such as anorexia, drowsiness, purplish comb, malaise and general indisposition, are also too general to be diagnostic of piroplasmosis and are common to many other poultry diseases.

While studying spirochaetosis of imported and local breeds of fowls in Sudan, Balfour [1907; 1908—1,2 1909 and 1911] encountered an 'after-phase' of the disease in birds from whose blood the spirochaetes had disappeared; this phase was characterized by the appearance in red blood corpuscles of rounded intra-corpuscular bodies, which, he thought, might be latent forms of spirochaetes. The nature of these bodies and their relationship to spirochaetosis have been the subject of much discussion, but they have since been definitely identified as a species of poultry piroplasm and in 1929 Carpano designated them as *Aegyptianella pullorum*. Earlier workers on avian spirochaetosis in India [Reaney, 1907 and Montgomery, 1908] do not seem to have encountered 'Balfour's bodies' (or an after-phase of the disease) but Knowles, Das Gupta and Basu [1927] recorded the occurrence of these bodies in birds recovered from experimental infection and also in healthy fowls purchased from the Calcutta bazar where the natural infection of the disease did not exist. These authors dismissed the intra-corpuscular bodies as products of 'Karyorrhexis of the erythrocyte nuclei, poisoned by the toxins of the disease' and the mortality apparently associated with the occurrence of these bodies was ascribed by them to 'crowding of the fowls in cages and much handling of them by sweepers and laboratory attendants.' In a more elaborate communication, these authors [1932] reported further observations in support of the foregoing theory and claimed to have produced similar bodies in the red blood cells by injections of toxic substances like phenyl hydrazine and benzyl benzoate. They did not, however, explain the spontaneous appearance of these bodies in the absence of spirochaetes and do not seem to have noticed Carpano's [1929] work. The work of Knowles, Das Gupta and Basu, however, led Hutyra, Marek and Manninger [1938] to consider the aegyptianellosis of fowls to be indigenous to India.

The present paper deals with the writer's observations on piroplasmosis of both imported and indigenous breeds of fowls in the Punjab.

Preliminary observations

At Hissar in May 1940, the writer was called upon to treat an one year old White Leghorn hen*, which was dull and off food since the previous day. On examination the bird was found to be samnolent, with a pale comb, ruffled feathers and body temperature at 108·0° F.; its droppings were watery and dark green in colour. A few larvae of *Argas persicus* were present under the wings and around the cloaca. Microscopic examination of a blood smear stained by Giemsa's method showed frequent tangles of *Spirochaeta anserinum*

* This bird and others of the same breed mentioned in the following pages belonged to a flock which had lived for several generations in India.

and rare intracorpuscular bodies, resembling those described by Balfour (*loc. cit.*) from Sudan; no anaemic changes were, however, noticed. A diagnosis of spirochaetosis was made and an injection of soamin (1·5 grain in 1·0 ml. of distilled water) was given in the pectoral muscles. The fowl house, in which this bird had been kept, was found to be heavily infested with *A. persicus*, in all stages of development. About 200 adult specimens of the tick were collected for transmission experiments, and the rest were destroyed in their natural habitat by means of a braziers' lamp. Two days after the injection, the hen was seen again and found to be bright and apparently normal; and its temperature had come down to 106·0°F. Microscopic examination of blood smears revealed no spirochaetes, but 'Balfour's bodies' were present as on the first day. A second intra-muscular injection of soamin (one grain in 1·0 ml. of distilled water) was given, and blood examined after the lapse of another day; 'Balfour's bodies' were still present as on the first day. On clinical grounds the bird was considered to have been cured.

Blood smears from some other adult birds of the same flock were examined and found to contain similar intra-corpuscular bodies in small numbers and un-accompanied by clinical symptons. Subsequent examination of the indigenous fowls at Hissar, Kasur and Lahore resulted in similar findings.

On closer examination the intra-corpuscular bodies were found to be of two kinds; (i) medium-sized to large round bodies and rings (1·2 to 4·0μ), which were present in small numbers—one in about ten fields, and which appeared to divide by breaking up into small granules (schizogony) in the erythrocytes; and (ii) small ring forms (0·7 to 1·5μ) which were somewhat more frequent than those of the first kind and which appeared to divide by budding or binary fission, one, two or four being in each erythrocyte. The larger bodies of the first type are morphologically indistinguishable from *A. pullorum*, and the smaller bodies of the second type bear a close resemblance to the haematozoon described by Coles [1937] from the American and African fowls, from which, however, they differ in that more than two may be present in a single erythrocyte, while their mode of division clearly differentiates them from smaller rings of *A. pullorum*. The specific identity of this parasite is being studied separately and in the rest of this paper it will be referred to as 'Coles' haematozoon.'

Transmission

The transmission experiment described below was carried out mainly to recover a strain of *A. pullorum* from ticks presumed to be infected with this parasite. For this purpose, a search was made at Hissar for clean fowls, but, at first, this search appeared to be quite futile because *Argas* was very common in the fowl houses. Later, however, a tick-free fowl house was found in which a large number of six to eight week old White Leghorn chickens were kept. Their blood was free from *A. pullorum* but a few 'Coles' haematozoa' were present in nearly all of them. In the absence of absolutely clean birds, two six-week old chickens of this lot were employed for the transmission experiment.

Two hundred adult fowl ticks (*A. persicus*), collected from an infected fowl house, were divided into three nearly equal groups and each group was kept overnight in a large glass jar with the two chickens, during three successive nights (2-5 June, 1940). A perforated cardboard disc placed at the bottom of the jar provided a platform for the chickens to stand upon and at the same time enabled the ticks to hide under it; the mouth of the jar was covered with a piece of muslin. On each succeeding morning numerous small blood clots were noticed on the legs of the chickens, indicating that the ticks had fed on them. Three days after the first batch of ticks had fed, one of the chickens (Chicken No. 2) received, intra-muscularly, 0·75 c.c. of blood direct from a hen showing fre-

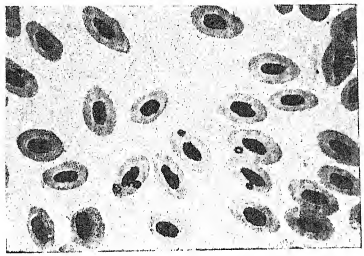

FIG. 1. *Aegytianella pullorum* in the blood of a chicken

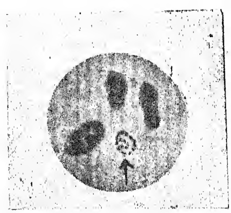

FIG. 2. Schizont of *A. pullorum* in a red cell in the blood of a chicken

quent *A. pullorum* in its blood.* Both these chickens were kept in a tick-free cage and their blood examined daily.

On 8, 9 June both the chickens were noticed to be somnolent, their temperature had risen to 109·2° F. and 108·4° F. respectively, and their blood showed frequent spirochaetes. The erythrocytes showed no *A. pullorum*, but 'Coles' haematozoa' were present in small numbers. After this, the chickens could not be examined until 12 June when they were found to be brighter and feeding well, their temperatures being 106·0° F. and 105·8° F. respectively. They had, however, become markedly emaciated and their combs were very pale. Blood examination showed neither spirochaetes nor *Aegyptianella*. They were considered to have recovered from the primary attack of spirochaetosis.

On 16 June (i. e. 14 days after the first batch of ticks had fed) the blood of both the chickens showed frequent *A. pullorum* with 'Coles' haematozoa' as before. A week later, *A. pullorum* became numerous in each field and continued to persist in large numbers until death. Symptoms and blood changes associated with this infection are mentioned below. The chickens in the flock, from which these two were taken, remained clinically healthy during this period and also during the rest of that summer.

The foregoing results are liable to be regarded as having failed to establish conclusively that *A. pullorum* was transmitted by *A. persicus*, inasmuch as the cage, in which the experimental chickens were kept, was not proof against other biting arthropods (e.g. mosquitoes) whilst, as already mentioned, one of the chickens had, in addition, received an injection of infected blood. However, in view of the experience of previous workers [Bedford and Coles, 1933] and the appearance of *A. pullorum* in peripheral blood after the lapse of an incubation period of 14 days, it seems highly probable that tick bites were the source of infection in these chickens.

* Subsequent observantions showed that this injection did not have any effect on the course of the disease in this chicken as compared to its companion.

Bedford and Coles [1933] have experimentally established that *Argas persicus* can transmit aegyptianellosis to healthy fowls with an incubation period of 12 to 15 days and that the ticks may remain infective for at least 162 days.

The presence of Coles' haematozoon in fowls living in tick-free fowl houses apparently shows that it is transmitted by an agent other than *Argas*. Coles [1937] also recorded this parasite from the vicinity of New York where, according to him, *Argas* was not likely to occur.

Symptoms and blood changes

It will be seen from what is stated above that piroplasms (*A. pullorum* and Coles' haematozoon) do not, as a rule, set up any appreciable symptoms in adult indigenous fowls or adult fowls of the White Leghorn breed which had lived for several generations in India. In the blood of these adult birds the piroplasms exist in small numbers. In the case of one bird an attack of natural spirochaetosis did not resuscitate the piroplasms; this disease was, however, treated quite soon after its appearance in the bird.

In the case of two six weeks' old White Leghorn chickens which showed *A. pullorum* following tick bites, clinical symptoms associated with the infection were studied. Both these birds first suffered from a mild attack of spirochaetosis which they survived without any treatment. *A. pullorum* first appeared in their blood 14 days after the first bites of infected ticks. At this time the only symptoms noticed were slight dullness and anorexia. Blood examination at this stage revealed 43 per cent of erythrocytes to be infected. A week later, sleepiness, dullness, emaciation and anaemia became well marked and the appetite capricious. The comb was quite blanched and the blood when drawn out of a needle prick was noticed to be distinctly watery. Body temperature showed a constant rise by 1·0° F. or so. Microscopic examination of blood revealed a heavy infestation of the red blood cells (60 per cent) with merozoits, small and

large rings, and 'rosette' forms of *A. pullorum*. The erythrocytes showed distinct polychromatia but it was notable that only the non-infected red cells had undergone this change. Coles' haematozoon was also present in small numbers and seemed to have undergone no apparent change in numbers as a result of the bites of infected ticks. The symptoms described above, particularly anæmia and emaciation, progressed till death occurred 27 days after the first appearance of *A. pullorum* in one case and 30 days in the other. Diarrhoea was noticed two days before death. No signs of paralysis were noticed in the legs or wings.

It may be mentioned that mortality from aegyptianellosis has been also recorded from many other countries. Carpano [1929] originally described ruffled feathers, fever, loss of appetite, drowsiness, paralysis and death as symptomatic of the disease among the imported fowls in Egypt. A fatal form of toe disease was also reported by Donatien and Lestoquard [1931] from Algeria, Gillain [1935] from Belgian Congo, Debonera [1933] from Greece and Yakimoff [1933] from the U. S. S. R. Coles [1933] described loss of appetite, dejection, diarrhoea and sometimes icterus as symptomatic of the disease in young chickens which had become infected during the first few days of life. Paralysis and fever reported by Carpano [1929] as characteristic of the disease were not noticed by the writer.

The factors governing the pathogenicity of *A. pullorum* do not appear to be properly understood, but age and immunity, possibly due to contact with *A. persicus* seem to play some part in its determination. Thus, fowls imported from countries free from *Argas*, and among them the younger birds, appear to suffer most.

Post-mortem appearances

The carcases of both the chickens showed similar anatomical changes. There was a well-marked thinness of muscles and general anaemia; the body cavity contained a slightly blood-tinged serous fluid and its walls were sparsely petechiated; the spleen was enlarged to about three times its normal size, its parenchyma being purplish in colour; the liver was also enlarged and appeared to be more friable; the pericardial sac contained a slightly blood-tinged fluid and the epicardium showed a few petechiae; and the alimentary canal showed nothing unusual. Smears of heart blood showed numerous *A. pullorum* (86 per cent erythrocytes were infected) and an almost similar infestation was found in smears from the lungs, liver and spleen. Polychromatia was noticeable in all the smears. Coles [1933] has mentioned icterus as one of the changes in his cases, but this change was not observed by the present writer.

Treatment

During the treatment of the first White Leghorn hen for spirochaetosis (*supra*) it was noticed that an intramuscular injection of soamin (1·5 grain) did not have any effect on *A. pullorum* or Coles' haematozoon, although the treatment caused the disappearance of *Spirochaeta anserinum*. A second injection of soamin (1 grain) given to the same hen two days later also failed to have any effect.

According to Hutyra, Marek and Manninger [1938], Stovarsol in doses of five centigrammes *per os* has a favourable effect on anaemia, although it does not kill the parasites (*A. pullorum*). Donatien and Lestoquard [1934] studied the effect of Stovarsol, Gonacrine, Trypan Blue and Icthargan on affected pullets. They found that Stovarsol was useful in cases of anaemia only and among the other drugs Icthargan was efficacious only after a second injection. In the hands of Curasson [1938], however, two intra-muscular injections of Gonacrine gave the best results. On the whole, therefore, the position regarding the chemotherapy of aegyptianellosis must be regarded as unsatisfactory.

Summary

1. The occurrence of *Aegytianella pullorum* Carpano and of a haematozoon

resembling that described by Coles [1937] in fowls has been recorded from northern India.

2. These haematozoa did not appear to set up any appreciable disease symptoms in the adult indigenous fowls or acclimatized imported breeds.

3. In an attempt to experimentally recover *A. pullorum* from naturally infected ticks (*Argas persicus*), two Leghorn chickens were exposed to the tick bites. These birds suffered from a mild attack of spirochaetosis on the 6th day and recovered; on the 14th day they, however, showed *A. pullorum* in their peripheral blood.

4. The symptoms shown by infected chickens were a progressive anaemia and emaciation, slight rise of temperature, dullness, diarrhoea and death in about four weeks' time. The main post-mortem findings were: the enlargement of the spleen and liver, petechiae on the serous membranes, slightly blood-tinged fluid in serous cavities and cachexia.

5. Coles' haematozoon did not appear to set up any disease symptoms in fowls and circumstantial evidence showed that this organism was probably transmitted by an agency other than the fowl tick.

6. Soamin, in the usual doses employed for the treatment of spirochaetosis, was found to have no effect either on *A. pullorum* or Coles' haematozoon.

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FEEDING VALUE OF FUZZY AMERICAN COTTON SEEDS AS COMPARED WITH DESI COTTON SEEDS FOR MILCH BUFFALOES

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There have been in the past and there still are to-day many prejudices amongst zemindars against using fuzzy American cotton seeds as a feeding stuff. These prejudices may briefly be summarized as follows:

1. Fuzzy cotton seed is low in fat in comparison with naked seed, and consequently the output of ghee from buffaloes fed on it is reduced.
2. This seed is considered to be indigestible or harmful to the digestive system of animals.
3. Fuzzy seed has a smaller kernel than other cotton seed and this reduces its nutritive value and adversely affects the fat content of the milk produced.
4. It reduces the quantity of milk secreted.
5. Some people go so far as to say that the teats of milch animals become clogged up if they are fed on this seed for any length of time.
6. Its food value is lower than that of other cotton seed.
7. Cattle do not relish the seed because of its fuzziness.

Work has been carried out at Lyallpur in the past on heifers with various types of cotton seeds including the fuzzy American types. The results of this work have been published in the following papers:—

(i) *Memories of the Department of Agriculture in India*, Chemical Series, Vol. X, No. 6, November, 1929.

(ii) *The Indian Journal of Veterinary Science and Animal Husbandry*, Vol. V, Part IV, December, 1935, pages 343-349.

The results of similar work carried out on milch cows are recorded in the progress report of the scheme for studying the nutritive requirements of milch cows in the Punjab for the year 1943.

These results indicate that none of the prejudices enumerated above can be substantiated as regards heifers and milch cows. Correspondingly those prejudices which might be expected to apply to working bullocks have similarly been shown to be without foundation. It is unfortunate that in spite of this work these prejudices still exist. One of the results is that the market price of fuzzy American cotton seeds has been, and still is, lower than that of naked *desi* seeds. This is a matter of considerable economic importance to cotton growers, and is one which has been prominently before the Indian Central Cotton Committee. This body recently considered it desirable that further work should be done on this problem using milch buffaloes in order finally either to dispel or to substantiate the prejudices in question. The Indian Central Cotton Committee therefore financed during the year 1942-43 a scheme of work which has been conducted at Lyallpur and which is described below.

Twelve milch buffaloes of, as far as possible, approximately equal weights, the same age and otherwise similar were used. These were divided into three groups of four each, designated as groups A, B and C. Group A was fed fuzzy American cotton seed, group B delinted American cotton seed and group C *desi* cotton seed. The work was started on November 1, 1942 and ended on 31 October 1943.

TABLE I

Particulars of the buffaloes

Animal Nos.	No. of Lactation	Date of calving	Date of service	Average body weight		Milk yield per lactation in lb.			
				Nov. '42	Oct. '43	I	II	III	IV
1	IV	23-9-42	18-6-43	1275	1271	4362	4790	5554	5654
2	,,	6-10-42	17-7-43	1282	1358	3823	4473	4166	3334
3	,,	29-9-42	22-8-43	1355	1379	4805	5480	5860	7207
4	,,	12-10-42	8-6-43	1254	1338	4725	4844	4261	5671
5	,,	23-9-42	15-5-43	1280	1482	3835	2459	5128	5053
6	,,	15-9-42	8-6-43	1220	1310	4503	4623	4445	5019
7	,,	18-9-42	30-8-43	1199	1239	1973	5319	4727	7681
8	,,	22-8-42	15-9-43	1295	1324	4165	3509	3215	4259
9	V	2-9-42	..	1209	1272	4738	4556	4094	3689
10	IV	15-9-42	..	1374	Died	3259	4757	5113	Died.
11	,,	3-9-42	2-4-43	1364	1396	..	3841	4700	4961
12	III	24-9-42	8-7-43	1226	1245	..	4191	..	5698

Table I shows particulars of the buffaloes, including the total milk yield per lactation in lactations Nos. I, II and III before they were purchased, and in lactation No. IV, during the experimental period. The animals arrived at Lyallpur on 29 October 1942, having calved at various dates in September 1942.

TABLE II

Average daily milk yield in lb. and average fat percentage of different groups of animals during different months of the year

Name of months	Milk Yield in lb.			Fat percentage		
	Group A	Group B	Group C	Group A	Group B	Group C
1942						
November ..	20·9	19·0	20·1	7·7	7·1	7·4
December ..	20·3	20·4	21·6	7·9	7·3	7·8
1943						
January ..	20·1	21·8	21·1	8·1	7·7	8·4
February ..	19·4	20·2	19·9	7·8	7·4	7·7
March ..	18·7	19·3	18·2	8·0	7·6	7·8
April ..	17·0	17·8	16·3	8·1	7·6	8·2
May ..	15·0	16·8	14·6	8·0	8·4	9·0
June ..	13·7	13·2	12·6	9·0	8·6	9·1
July ..	12·5	11·3	10·7	9·4	8·9	9·4
August ..	13·4	10·6	9·8	9·4	9·1	9·6
September	10·7	8·4	6·6	9·4	9·2	9·6
October ..	8·5	8·7	4·6	9·4	8·8	9·2

Group A=289F (fuzzy cotton seed)
Group B=289F (delinted cotton seed)
Group C=*Desi* cotton seed

Table II shows the average daily milk yield in lb. and average fat percentage of different groups of animals during different months of the year.

Reference to Table I shows that during the period of the trials the body weights of the animals remained quite normal. Reference to Table II shows that with one or two exceptions the milk yields during the experimental lactation period were greater than during the previous lactations (Buffalo No. 2 fell sick on arrival at Lyallpur and later on her calf died, hence the fall in total yield of milk, and buffalo No. 9 was in calf when purchased and ceased giving milk in the month of May). Two conclusions may thus immediately be drawn, namely that neither the body weights of the animals nor the milk yields suffered.

The first line of attack was to ascertain the maximum amount of the various types of cotton seeds which the animals would eat, and to correlate the figures obtained with the seven contentions set out above, and to study the ghee produced from the milk.

Group A was fed K25/289F fuzzy American cotton seed.

Group B was fed K25/289F delinted American cotton seed.

Group C was fed *desi* cotton seed.

During the experimental period all the animals received the same green fodder and

TABLE III

Average results of the analyses of the ghee (butter fat) prepared during different months of the year from different groups of buffaloes

Month of analysis	Reichert Meissl value			Polenske value			Kirschner value			Saponification value			Iodine value			Refractive index		
	289F (fuzzy cotton seed)	289F delinted seed	Desi cotton seed	289F (fuzzy cotton seed)	289F delinted seed	Desi cotton seed	289F (fuzzy cotton seed)	289F delinted seed	Desi cotton seed	289F (fuzzy cotton seed)	289F delinted seed	Desi cotton seed	289F (fuzzy cotton seed)	289F delinted seed	Desi cotton seed	289F (fuzzy cotton seed)	289F delinted	Desi cotton seed
1942																		
November	34·86	32·22	29·65	2·4	1·9	2·0	29·80	28·31	26·58	230	227	222	27·8	27·9	29·3	1·4539	1·4544	1·4542
December	34·10	31·68	29·50	2·1	1·7	2·0	29·25	27·10	25·00	228	224	221	28·1	28·4	30·2	1·4550	1·4545	1·4545
1943																		
January	33·80	31·25	28·70	1·9	1·7	1·6	28·50	27·40	25·29	230	227	222	28·6	29·8	31·1	1·4550	1·4545	1·4548
February	31·26	29·25	26·00	1·9	1·4	1·8	27·90	24·95	22·70	229	224	220	29·8	30·0	29·8	1·4544	1·4549	1·4550
March	30·60	29·05	25·0	1·6	1·4	1·1	27·70	24·10	22·00	225	223	219	31·1	30·1	30·5	1·4547	1·4549	1·4554
April	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..
May	29·70	26·50	23·50	1·4	1·1	1·0	27·05	23·07	20·50	225	222	219	31·9	31·2	32·0	1·4541	1·4553	1·4558
June	28·76	24·43	23·33	1·7	1·1	1·7	26·35	22·01	20·70	224	219	217	33·9	34·9	36·1	1·4547	1·4553	1·4551
July	26·60	23·50	20·15	1·4	1·4	1·5	23·56	21·42	20·21	222	219	216	35·3	35·8	37·8	1·4547	1·4549	1·4551
August	23·06	21·07	18·74	0·9	1·1	..	21·36	18·96	18·18	222	218	217	37·7	38·9	40·8	1·4551	1·4551	1·4551
September	27·10	25·14	22·89	1·3	1·4	1·1	23·79	21·76	18·88	227	225	219	35·1	34·6	36·9	1·4535	1·4535	1·4551

dry roughage. Maize fodder was given at the rate of 130 lb. per head per day with about 5 lb. of wheat *bhusa* during November and December, 1942. From January to May, green berseem was fed and then till the end of the experiment, maize. The amount of *bhusa* given per day remained about the same. In addition to the above-mentioned roughages, all the animals were given as much cotton seed as they could eat together with 1 lb. of ground gram and 1 lb. of wheat bran per head per day. The four buffaloes in group A ate regularly approximately 6 lb. per head per day of cotton seed throughout the period. Of the four buffaloes in group B, two ate 8lb. of the delinted cotton seed (K25/289F American cotton seed) per head per day whilst the other two would not eat more than 4 lb. per head per day. The animals in group C ate up to 8 lb. of the seed per head per day on an average although animal No. 11 ate as much as 14 lb. of *desi* cotton seed per day for about six weeks. One animal in group C, No. 10, unfortunately developed mammitis in November, 1942, and died on 16 January 1943, of toxemia. With this exception, all the animals maintained normal weight with slight increase throughout the period under report. Their health was excellent and the milk yield in every case normal.

All three types of cotton seed were soaked in water for half an hour or so before being fed.

Comments on the nature of the ghee produced will be given later. A sample of ghee was made from the milk of each animal each month during the course of the experiment except April 1943, when the buffaloes were inoculated against T. B. The results of the analyses are shown in Table III.

As a result of these and previous investigations the following conclusions may be drawn on the seven contentions mentioned above:

1. The statement regarding a lower fat content of fuzzy cotton seed is not correct, as may be seen from the statement of chemical analyses of the various cotton seeds (Table IV). There is no truth in this contention and the output of milk or ghee from buffaloes fed on fuzzy cotton seed was not reduced.
2. The fact has been deduced from these trials that fuzzy cotton seed is more digestible and contains more total digestible nutrients and more digestible protein per 100 lb. of seed than naked cotton seed.
3. From what has been said above, this is obviously not true.
4. From the records of the milk yields, it will be seen that there is no truth in this contention.
5. In regard to this contention, one of the animals in group A suffered from slight mammitis for a short time but completely recovered, although the feeding régime remained the same. On the other hand, one of the animals in group C suffered and ultimately died from toxemia. Mammitis is not an uncommon occurrence and may occur in any animal, and in this case appears to have had no traceable relationship with the ration.
6. It has already been pointed out that in these experiments the food value of the fuzzy cotton seed was higher than that of the other cotton seed, and not lower. This fact had previously been ascertained by work on heifers and from the results of similar works carried out on milch cows recorded in the progress report of the scheme for studying the nutritional requirements of the milch cows in the Punjab for the year 1943.
7. This is entirely unsupported from the evidence gained in these experiments and previous work on milch cows and heifers.

TABLE IV

Chemical composition of various cotton seeds

	Moisture per cent	Dry matter per cent	Ash per cent	Fat per cent	Fibre per cent	Protein per cent	Nitrogen free extract per cent	Total digestible nutrients in lb.	Digestible protion in lb.	Albuminoid
At the beginning of the experiment										
1. Fuzzy American cotton seed K 25/289F.	7·0	93·00	4·63	19·28	26·50	16·38	26·21	75·26	10·12	6·1
2. Delinted American cotton seed K 25/289F.	6·50	93·50	4·87	18·70	22·90	17·70	29·33	73·70	10·80	5·9
3. Desi cotton seed	6·98	93·02	4·93	17·54	21·50	15·80	34·05	73·90	8·10	8·6
At the end of the experiment										
1. Fuzzy American cotton seed K 25/289F.	5·90	94·10	4·80	19·30	26·52	16·40	27·08	75·26	10·12	6·1
2. Delinted American cotton seed K 25/289F.	5·50	94·50	4·90	18·70	22·95	17·70	30·15	73·70	10·80	5·9
3. Desi cotton seed	6·00	94·00	4·95	17·54	21·50	15·20	34·81	73·90	8·10	8·6

The above discussion should set at rest popular objections, and it indicates that there is no valid reason for the prejudice which gave rise to the conduct of these trials. Some people may still opine that the taking into an animal's digestive tract of a considerable amount of lint every day must be deleterious, but they overlook the fact that the animal's digestive system deals with it and digests it, and in any case the experimental animals definitely did not suffer in any way.

The technical aspects relating to the ghee produced from the milk may be considered under two headings:

1. The general impressions formed concerning the ghee produced from the milk of buffaloes fed on cotton seed by those who have used it.
2. The scientific data relating to the composition of the ghee in comparison with the standard specifications laid down by Government.

In regard to the quality of the milk, ghee and butter produced from the fuzzy cotton seed fed group, it has not been possible to distinguish, by consumption, any difference in taste or palatability from similar produce made from the milk of buffaloes fed different rations.

In March 1943, samples of ghee and butter made from the milk of American

TABLE V

Summary of opinion of members of the Indian Central Cotton Committee on the samples of ghee

Sample A=desi cotton seed fed animals

Sample B=fuzzy cotton seed fed animals.

Gill & Co., Bombay ..	Ghee and butter are outstanding and flavour superior
Sir Porshotam Das Thakurdas, Bombay	No difference in them
Sir Sorab Saklatvala, Bombay	Both the samples of ghee are good
Rao Bahadur Thadani, Director of Agriculture, Sind	Both the samples of ghee and that of butter are good. Feeding of American cotton seed does not produce any deterioration in quality of ghee and butter
S. R. Pocock & Co., Cawnpore	Sample B considered better
Sir Chuni Lal V. Mehta, Bombay	Sample A better than sample B. Butter smell not good. The bottle containing butter got damaged during transit
Sir Chuni Lal B. Mehta, Bombay	No tangible difference between the two samples. A gave better taste. B gave unusual smell
C. Maya Das, Director of Agriculture, U. P.	Sample A better. B too oily

fuzzy cotton seed fed animals and *desi* seed fed animals respectively were sent to 20 members of the Committee to afford them an opportunity to form their own opinion. Eight members replied, and the general consensus of opinion summarized in Table V shows that the ghee from the fuzzy cotton seed fed animals was preferred. The samples were despatched to certain parts of the country when the weather was becoming warm and in some cases the butter did not arrive in as sound a condition as might have been hoped for. This was perhaps the result of faulty packing. The balance of evidence was in favour of the fuzzy cotton seed.

SCIENTIFIC DATA REGARDING THE GHEES

Table III shows the complete analysis of the ghee made from the milk of the respective groups of animals throughout the year. In March 1943, a composite sample of ghee made from the milk of the animals of each of the three groups was sent to Sir Sorab Saklatvala of the Tata Oil Mils, Bombay. Their Chemist found that his Polenske values were somewhat lower than the corresponding Lyallpur figures. In this connection it is interesting to note the correlation which should exist between the Reichert Meissl values and the Polenske values as given on page 379 of Vol. II, Oils and Fats of Allen's *Commercial Organic Analysis* and the corresponding correlations in these values as seen from the Lyallpur analysis and the Tata analysis respectively.

The analysis of the ghee sent showed certain deviations from the Government specifications, which have been kept within rigid limits to safeguard the public. There appeared to be nothing in the analytical figures to show that the ghee made from the milk of the fuzzy and naked cotton seed fed groups of animals depart from normal to any extent which would constitute an argument against either.

Summing up, these trials have shown that the prejudice against fuzzy cotton seed is unfounded, and the buffaloes fed on the rations indicated did not suffer

TABLE VI

Correlation between Reichert Meissl values and Polenske values

Allen's commercial organic analysis		Lyallpur figures		Figures obtained by the chemist of the Tata Oil Mill	
Reichert Meissl values	Polenske values	Reichert Meissl values	Polenske values	Reichert Meissl values	Polenske values
32	3·5	35	2·4	31	1·2
31	3·2	34	2·1		
30	3·0	34	1·9		
29	2·9	31	1·9		
28	2·7	31	1·5		
27	2·4	30	1·4		
26	2·0	29	1·7		
25	1·8	27	1·4		
24	1·7	23	0·9		
23	1·6	27	1·3		

on that account (the animal which died was a *desi* cotton seed fed animal) and neither the quantity nor quality of the milk or ghee depart from normal to any extent which could conceivably be held to be an objection. This evidence, and the published record of previous work on cows with cotton seed and cotton seed cake should be brought to the notice, in some authoritative way, of the zamindar community in India.

The authors wish to take this opportunity of thanking the Indian Central Cotton Committee for providing the funds for this work.

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PROTEINS FOR CHICKENS

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In earlier experiments, Macdonald [1943] concluded that meat offal (intestines) can be used as an effective substitute for milk in the ration of chickens. As in many parts of the country separated and other milk products are relatively expensive, it was decided to carry out a further comparison in order to test the relative values of meat offal and separated milk. As cereal diets without protein supplements normally result in high mortality when fed to young chickens, and, as in previous experiment, a cereal diet supplemented with salt, green food and calcium gave comparatively low mortality, the new experiment was designed to throw more light on the value of common salt in the diet of birds fed on low protein diets.

Methods

Stock. A total of 296 White Leghorn × Rhode Island Red chickens hatched in November 1942 were divided into four comparable groups and fed on the experimental diets for a period of 20 weeks.

Feeding. All the birds were fed on a basal mash consisting of wheat bran 50 parts, yellow maize meal 30 parts and ground oats 20 parts. During the first eight weeks, the birds were fed a grain ration of equal parts maize, *jowar* and *cheena*. From 8-20 weeks the grain ration consisted of equal parts yellow maize, wheat and paddy. The grain was fed at 7 a.m. and 4-30 p.m. throughout the experiment. Broken limestone was supplied *ad libitum* and succulent green food (berseem) was fed liberally once daily during the whole experimental period. The mash was fed daily in a wet, crumbly state according to appetite at 9 a.m., 12 noon and 3 p.m. Group I was fed the basal ration only. Group II received the same ration as Group I with the addition of 1 per cent common salt to the mash. Group III was fed the basal mash plus 0.5 per cent salt and the mash made up with separated milk instead of water. Separated milk was also given *ad libitum* during the first 12 weeks, whilst separated milk and water in separate containers were supplied from 12-20 weeks. Group IV was fed the basal mash plus 1 per cent common salt and meat offal (cleaned intestines from the slaughter house). The amount of meat offal fed was regulated, so that the protein content of the ration was approximately the same as that fed to the birds receiving separated milk (Group III). The quantity of meat offal required every day was cooked for one hour in a small quantity of water and run through a mincing machine prior to mixing with the mash. The minced meat offal and the liquid residue after cooking were mixed with the required daily amounts of mash and equal quantities were fed at each time of feeding.

Rate of growth. The chickens were weighed individually at fortnightly intervals. The average weights of the males and females from 1-9 weeks and the females from 11-21 weeks are given in Table I. The cockerels were removed from the experiment at the end of the second period (9 weeks).

TABLE I

Average weights in ounces and probable errors

Sex	Group I Male	Group I Female	Group II Male	Group II Female	Group III Male	Group III Female	Group IV Male	Group IV Female
1 weeks	2.0 ±0.06	1.9 ±0.02	2.0 ±0.05	1.9 ±0.03	2.0 ±0.03	1.9 ±0.02	1.9 ±0.05	1.9 ±0.03
3 weeks	3.1 ±0.06	2.9 ±0.08	3.4 ±0.09	3.3 ±0.07	4.1 ±0.19	3.7 ±0.13	4.3 ±0.15	3.9 ±0.19
5 weeks	4.0 ±0.11	3.4 ±0.12	4.3 ±0.15	4.1 ±0.08	8.6 ±0.29	7.5 ±0.20	8.2 ±0.20	7.2 ±0.19
7 weeks	5.6 ±0.11	4.8 ±0.12	5.4 ±0.15	4.8 ±0.08	13.2 ±0.29	11.2 ±0.20	13.2 ±0.20	10.6 ±0.19
9 weeks	7.4 ±0.19	6.5 ±0.15	7.6 ±0.27	6.9 ±0.16	20.6 ±0.49	17.4 ±0.26	17.7 ±0.51	14.0 ±0.30
11 weeks		9.3 ±0.25		9.9 ±0.21		22.7 ±0.31		19.0 ±0.50
13 weeks		12.9 ±0.40		13.0 ±0.26		29.0 ±0.34		26.2 ±0.52
15 weeks		17.1 ±0.40		17.1 ±0.40		34.9 ±0.68		31.9 ±0.62
17 weeks		22.4 ±0.48		21.4 ±0.34		38.6 ±0.61		36.0 ±0.64
19 weeks		27.6 ±0.51		26.6 ±0.50		43.0 ±1.00		41.8 ±0.75
21 weeks		33.1 ±0.59		32.7 ±0.51		46.8 ±0.89		45.5 ±0.69

At 9 weeks the average weights in onces of the males in Groups I-IV (Table I) were 7.4, 7.6, 20.6 and 17.7 respectively. The corresponding figures for the females were 6.5, 6.9, 17.4 and 14.0. At 21 weeks the average weights in ounces of the pullets in Groups I-IV were 33.1, 32.7, 46.8 and 45.5 respectively. At five weeks and at each subsequent weighing, the birds in Groups III and IV were significantly heavier than those in Groups I and II. At no period was there any significant difference between the average weights of Groups I and II or between those of Groups III and IV.

Food consumption. Table II gives the average food cousumption in pounds per bird for each group for each period of four weeks and the corresponding food consumption figures in pounds per pound of live weight gain. For comparative purposes, the figures for the meat offal and milk were brought to a dry matter basis and the resultant figures added to the mash and grain consumptions.

TABLE II

Food consumption

Serial No.	Period	Av. food consumption per bird (lb.)				Food consumption in lb. per lb. live weight gain			
		Group I	Group II	Group III	Group IV	Group I	Group II	Group III	Group IV
1	1-5 weeks ..	1.14	1.13	1.57	1.42	9.11	7.95	4.15	3.96
2	5-9 weeks ..	1.40	1.38	2.73	2.45	6.33	7.15	4.06	4.75
3	9-13 weeks ..	2.28	2.08	4.71	4.45	6.67	5.66	6.68	6.16
4	13-17 weeks ..	4.71	4.31	5.54	5.33	9.24	8.47	8.79	8.37
5	17-21 weeks ..	6.60	5.76	6.56	6.65	11.09	10.30	12.44	11.83

During the first two periods the average food consumption per bird was very similar in Groups I and II but in periods 3-5 the birds in Group I consumed somewhat more food than those in Group II. During periods 1-4, the birds in Groups III and IV consumed considerably more food than those in Groups I and II. During period 5, the average food consumptions were very similar for Groups I, III and IV and higher than those for Group II. During the first two periods, Groups III and IV made very much better utilization of the food consumed than Groups I and II. During the subsequent three periods, the efficiency of food utilization in all the four groups was fairly comparable.

Protein consumption. Table III gives a summary of the average protein percentages in the foods consumed by each group for each of the five periods.

TABLE III

Percentage protein consumed

Serial No.	Period	Group I	Group II	Group III	Group IV
1	(1-5 weeks) ..	10.9	10.9	16.0	16.8
2	(5-9 weeks) ..	10.9	10.8	16.0	16.5
3	(9-13 weeks) ..	10.8	10.8	14.5	15.2
4	(13-17 weeks) ..	10.8	10.8	13.6	13.8
5	(17-21 weeks)	10.8	10.8	12.7	13.0

The percentage protein consumption figures for Groups I and II are practically identical throughout and are in all cases considerably lower than those recorded for Groups III and IV. The protein consumption figures for Groups III and IV are fairly comparable throughout but on the average the protein level was slightly higher for Group IV. The percentage of protein in the food consumed by Group III started off at an initial level of 16.0 in period 1 and fell to 12.7 per cent in period 5. The corresponding figures for Group IV were 16.8 and 13.0.

Mortality. Table IV gives a statement of mortality percentages for each period and the total mortality in each group for the whole experiment.

TABLE IV,

Mortality percentages (1-21 weeks)

Period	Group I	Group II	Gronp III	Goup IV
1.	1.35	..	1.35	..
2.	4.05	4.05	1.35	4.05
3.	7.69	7.02	..	..
4.	11.90	1.92	..	..
5.	..	1.92	..	..
Total	24.99	14.91	2.7	4.05

The general health of the birds in Groups I and II as judged by handling and appearance was consistently poorer than that of Groups III and IV. The general health of the birds in Groups III and IV was consistently good throughout the experiment. The total mortality percentages in Groups I and II were much higher, being 24.99 and 14.91 respectively, while the corresponding figures for Groups III and IV, were 2.7 aud 4.05 respectively.

DISCUSSION

The rate of growth of the birds in both the groups without protein supplements was very much lower than that of the two groups receiving the protein supplements. The slow rate of growth in Groups I and II was most marked during periods 1 and 2 (1-9 weeks), so in the period when the demand for protein is at a maximum. The food utilization figures for the non-protein supplemented groups during periods 1 and 2 were much inferior to those in the corresponding periods for the protein supplemented groups. Throughout the whole experiment the general health and appearance of the birds in Groups I and II were much inferior to those of Groups III ahd IV. The mortality in all groups was low until the end of the second period. As would be expected with chickens reared under good environmental conditions, no death occurred in the protein supplemented groups for 9-21 weeks. On the other hand, Groups I and II both suffered substantial mortality from nine weeks onwards. The low mortality in Groups I and II during the initial eight weeks of the experiment period seems rather surprising in view of the low protein content of the ration. The low mortality during this period can partly be attributed to all the birds having received well balanced rations for one week prior to commencing the experiment. Furthermore, during this period of eight weeks, the birds were well protected against the weather and thus did not receive any further check except that imposed by the diets fed. From 9-21 weeks, however, all the groups were placed outside in houses which were more exposed to weather conditions and this, in addition to the cumulative effect of deficient feeding in

the case Groups I and II, resulted in a certain amount of mortality.

The results obtained with the low protein diet in this experiment as regards rates of growth, food utilization and mortality are very much better than those reported by Macdonald [1941] on a similar low protein diet. In this experiment, however, the birds received a good initial diet from 0-1 week and it is very probable that this good start might have exerted a very beneficial influence on the subsequent behaviour of the chicks.

The rate of growth and food utilization figures for Groups I and II were very similar. Whilst Group II had lower mortality than Group I, it would be unsafe to conclude from this that the addition of salt lowered mortality, since both groups were similar in appearance at all stages. The failure of the addition of salt to give any response is somewhat surprising in view of the proved value of salt supplements in rations containing only vegetable proteins. Mitchell and Carman [1926] demonstrated that the addition of common salt to a diet mainly composed of maize greatly improved its value. Prentice and Baskett [1932] and Prentice [1933] found that salt was very beneficial in the diet of chickens. Robertson, Orr, Prentice and Macdonald [1930] demonstrated that a supplement of salt was very beneficial in diets containing vegetable proteins only. In this experiment, however, the protein level of the ration was lower than that in the experiments reviewed here. Furthermore, the liberal feeding of berseem, which is relatively rich in both sodium and chlorine, should have helped to make good any deficiency of both sodium and chlorine in the cereals. Robertson, Orr, Prentice and Macdonald [1930] also reported the beneficial value of clover in a vegetable protein diet containing no salt supplement.

During the major part of the experiment, the chickens on the separated milk diet were slightly heavier than those on the meat diet. On the other hand, the meat group made on the average slightly better utilization of the food consumed. As in previous work, Macdonald and Bose [1943] obtained slightly better growth results from meat offal, further experiments will have to be carried out prior to making any final conclusion in regard to the relative values of separated milk and meat offal in the diet of growing chicks. As the unit of protein in meat offal is normally cheaper than that in separated milk it is, however, safe to conclude that meat offal could often be profitably used in place of milk in the diet of chikens.

Conclusions

(1) A mixed cereal diet with a protein content of 10.8 per cent plus liberal amounts of green food and calcium is unsatisfactory for growing chickens, especially in the early stages of growth.

(2) Little or no benefit is derived by supplementing the above diet with common salt.

(3) Separated milk and meat offal are valuable protein supplements for chickens.

(4) Meat offal can often be used very profitably as a substitute for separated milk in the diet of chicks.

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STUDIES IN SANITIZING DAIRY UTENSILS UNDER INDIAN CONDITIONS

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Introduction

Milk, which is a valuable human food, provides a favourable medium for the growth of micro-organisms. The Bacteria on entering the milk multiply at a rapid rate if the temperature is favourable to them, and bring about undesirable chemical changes, as a result of which the keeping quality of the milk is considerably impaired. It, therefore, becomes imperative to take proper precautions against its contamination from all possible sources during handling. One of the most common sources of contamination of milk is the utensil used in its production and handling, if it is not properly cleaned and sterilized. Rowlands [1934] has shown that ropiness in milk can be spread by churns (large milk cans) which are not properly sterilized. Barkworth [1941] has given the following figures indicating the extent of contamination of good quality milk through dirty churns:

Milk in the bucket	..	12,000 bacteria per ml.
Same milk in churn (dirty)	..	9,900,000 „ „ „

It is, therefore, evident from the above that the sanitizing of milk utensils is important not only from the point of view of enhancing the life of milk, but also to supply milk of a low bacterial count to the consumer. Investigations by Kelly [1914], Prucha and Harding [1924], Passon [1926], Theophilus and Atkeson [1931], Yale [1935] and Burgwald [1939] have indicated the importance of clean and sterile utensils in the production and handling of high quality milk.

Extensive research has been carried out in the past to find out suitable detergents for cleansing dairy utensils and equipments to render them sterile enough for the safe handing of milk for all practical purposes. Some of the common detergents that are highly alkaline include caustic soda, soda ash, washing soda, sodium metasilicate, trisodium phosphate and sodium hexametaphosphate. Mixtures of these are sold under a variety of trade names such as 'Dairy detergents, or Washing powders.' Washing or cleaning of milk utensils with these detergents, followed by sterilization with steam or scalding with boiling water, is a common practice in most of the up-to-date dairies. Farral [1929], Barker [1929], Rogers and Evans [1936] and Provan and Treble [1941] have shown that the cleaning of dairy utensils and equipments with alkaline washing compounds and the application of steam render them practically sterile.

Although much work has been carried out in Western countries on the cleaning and sterilizing of dairy equipment, no published data are available in India excepting the investigation carried out by Verma, Paul and Kothavalla [1941] who have shown that the comparative detergent efficiency of soda ash, wood ash and mud on milk utensils is in the descending order. Due to war the prices of washing soda and other dairy detergents have greatly increased and it, therefore, became necessary to investigate the possibilities of finding a suitable substitute for them and to evolve a cheap and efficient method of cleaning dairy utensils. In India the villagers use mud, wood ash, tamarind and mud or ash and mixture of mud and cow-dung cake ash for cleaning their milk utensils. The last mentioned method (mixture of mud and cow-dung cake ash) is generally employed, followed by an exposure to the sunlight. The bactericidal potency of this method, however, has not so far been studied systematically.

Recently a product known as Bentonite (a kind of clay) was brought on the market, and it was claimed for it that it was a useful detergent for cleaning dairy utensils and cheaper* than washing soda. When Bentonite was tested in the laboratory it was found to give alkaline reaction to phenolphthalein and contained traces of carbonates as indicated by the slight effervescence it gave with hydrochloric acid and appearance of white turbidity when barium chloride solution was added to clear water extract of Bentonite. The following composition of Bentonite was supplied by the firm:

Moisture	.. 15.36	per cent.
Ignition loss	.. 7.24	,, ,,
Silica (SiO_2)	.. 49.53	,, ,,
Alumina (Al_2O_3)	.. 19.41	,, ,,
Iron oxide (Fe_2O_3)	.. 0.59	,, ,,
Calcium oxide (CaO)	.. 2.62	,, ,,
Magnesium oxide (MgO)	5.11	,, ,,
Sodium oxide (Na_2O)	.. 0.12	,, ,,
Potassium oxide (K_2O)	0.03	,, ,,
	100.01	per cent

In view of its alkaline property and low organic matter content, Bentonite indicated the possibilities of its being used as a dairy detergent suitable for conditions prevailing in this country. This investigation was therefore undertaken to determine its quality as a detergent when compared with washing soda, the commonly used detergent in the organized dairies. The village method of cleaning milk utensils with mud and cow-dung cake ash termed as 'indigenous method', has also been investigated for its bactericidal efficiency as compared with the method adopted in dairies, namely cleaning with washing soda using hot water for rinsing purposes, followed by steam sterilization. For the purpose of investigation the milk utensils used were of two types, namely bottles of 1 lb. and 2 lb. capacity and cans (brass tinned and untinned) of 1½ gallons capacity.

*Price of Bentonite .. Rs. 7-8-0 per cwt.
Market price of washing soda .. 25-0-0 ,, ,,

Experimental

Method of cleaning. The methods employed for cleaning milk bottles and cans with washing soda, Bentonite and indigenous method were as follows:

(*i*) *Milk bottles.* Sterile bottles were used so as to bring the initial contamination to the same extent in all cases. A measured quantity of raw milk was then put into these bottles and kept overnight to curdle. The following morning the curdled milk was drained off from the bottles, the bottles rinsed with tap water to remove the remnants of curdled milk and then cleaned with the three detergents as follows:

(*a*) *Washing soda.* The bottles were washed with 1 per cent solution of washing soda in hot water (140°F) and scrubbed with a sterile bottle brush. After this they were rinsed with hot water to remove the traces of alkali and then sterilized in a steam chest for three minutes.

(*b*) *Bentonite.* The procedure followed was the same as above.

(*c*) *Indigenous method.* After rinsing the bottles with tap water the mixture of mud and cow-dung cake ash in equal amounts were applied to them both inside and outside and the inside then scrubbed thoroughly with a sterile brush. The bottles were then rinsed with tap water till all the traces of mud particles were removed. Finally they were rinsed with water and kept exposed to sunlight for ½ hour first with their mouths down and then with their mouths and inside portions fully exposed to direct sunlight for an hour.

(*d*) *Controls.* The bottles were simply rinsed with tap water and scrubbed with a sterile brush till all the curdled milk particles were removed. Finally they were rinsed with tap water.

(*ii*) *Cans.* To start with all the cans were thoroughly cleaned with washing soda and then steam-sterilized for five minutes along with their lids. The initial cantamination of these cans when examined bacteriologically showed that their plate counts varied from 100 to 400 and the coliform organisms were absent in one ml. of the rinsed out solution in all the trials. A measured quantity of raw milk was then put into each of these cans and kept overnight to curdle. The rest of the procedure adopted in cleaning the cans by the different methods was the same as that followed in the case of bottles excepting that the cans and their lids were steam sterilized for four minutes after cleaning with washing soda and Bentonite. Sterile coconut coir was used for scrubbing the cans.

BACTERIOLOGICAL EXAMINATION

(*i*) *Bentonite.* Plate counts showed 56,000 bacteria per gram of Bentonite.

(*ii*) *Mixture of mud and cow-dung cake ash.* The bacterial counts varied from 12 to 15 millions per gram of the mixture (mud and cow-dung cake ash) used.

(*iii*) *Tap water.* The tap water used for washing the bottles and cans gave 640 colony counts per ml.

(*iv*) *Bottles and cans.* The technique employed for determining the bacterial counts of bottles and cans was the same as that suggested by Mattick and Hoy [1937] with the exception that saline solution was used instead of Ringer's solution for rinsing the utensils. In the case of cans 100 ml. of saline water were used for rinsing. Presumptive coliform test was done by introducing one ml. of the rinsed out solution into 10 ml. of MacConkey's bouillon.

The maximum, minimum and average counts of bottles and cans and the number of trials in eah case falling between different ranges of counts are give in Table I.

DISCUSSION

The foregoing results show that the bacterial counts of the bottles treated with washing soda and Bentonite are not much different and they are less than those of the bottles cleaned by the indigenous method. The counts of the bottles cleaned by the indegenous method are less than those of the controls. With the washing soda the counts of the bottles range from 0 to 190, with an average count of 95, and from 150 to 280, with an average count of 226, in the cases of 1 lb. and 2 lb. bottles respectively. The bacterial counts of bottles cleaned with Bentonite vary from 0 to 190, the average count being 85, in the case of 1 lb. bottles and from 150 to 280, with an average count of 230, in the case of 2 lb. bottles. The counts of the bottles obtained by the indigenous method range from 130 to 2,000, with an average count of 504 in the case of 1 lb. bottles and from 1,100 to 4,700, the average count being 3,034 with 2 lb. bottles. The plates count of the control bottles vary from 2,000 to 50,000 with an average count of 9,410 with 1 lb. bottles and from 5,300 to 75,000, with an average count of 18,069, in the case of 2 lb. bottles. Coliform is absent in all the trials with washing soda and Bentonite, but in the case of indigenous method it is present in 11 trials with 1 lb. and 2 lb. bottles. All the controls show positive test for coliform. The American Standard Methods [1939] recommend a maximum of 1, 000 colonies per quart (American) bottle and of 500 per pint bottle, whereas Mattick and Hoy [1937] suggested that 200 organisms per pint bottle should be the standard. All the results with 1 lb. and 2 lb. bottles cleaned with washing soda and Bentonite therefore attained these standards. In the case of indigenous method 16 trials with 1 lb. bottles attained American standards and only six trials came up to the standard proposed by Mattick and Hoy. All the trials with 2 lb. bottles cleaned by the indigenous method and the controls, however, failed to attain these standards.

Table I

Particulars	Experiments with 1 lb. bottles				Experiments with 2 lb. bottles				Experiments with 1½ gallon cans							
									Washing soda		Bentonite		Indigenous method		Control	
Total bacterial Counts	Washing Soda	Bentonite	Indigenous Method	Control	Washing Soda	Bentonite	Indigenous Method	Control	B.T. cans	B.U.T. cans	B.T. cans	B.U.T. cans	B.T. cans	B.U.T. cans	B.T. cans	B.U.T. cans
Maximum ..	190	190	2000	50000	280	280	4700	75000	2000	1900	2000	2000	9800	14000	300000	380000
Minimum ..	0	0	130	2000	150	150	1100	5300	300	300	200	100	900	700	6200	21000
Average ..	95	85	504	9410	226	230	3034	18069	1094	1161	1138	1166	366	6083	130122	155472
No. of trials.																
100 and below bacteria ..	16	19	0	0	0	0	0	0	0	0	0	0	0	0	0	0
101- 200 „	14	11	6	0	6	6	0	0	0	0	1	0	0	0	0	0
201- 300 „	0	0	11	0	17	17	0	0	1	3	2	1	0	0	0	0
301- 500 „	0	0	5	0	0	0	0	0	2	1	2	3	0	0	0	0
501- 1,000 „	0	0	4	0	0	0	0	0	3	3	3	2	1	1	0	0
1,000- 2,000 „	0	0	4	1	0	0	3	0	12	11	10	11	3	2	0	0
2,001- 4,000 „	0	0	0	3	0	0	19	0	0	0	0	0	5	2	0	0
4,001- 6,000 „	0	0	0	21	0	0	1	3	0	0	0	0	3	7	0	0
6,001- 10,000 „	0	0	0	1	0	0	0	6	0	0	0	0	6	4	1	0
10,001- 20,000 „	0	0	0	0	0	0	0	11	0	0	0	0	0	2	1	0
20,001- 50,000 „	0	0	0	4	0	0	0	0	0	0	0	0	0	0	5	6
50,000- 100,000 „	0	0	0	0	0	0	0	3	0	0	0	0	0	0	1	0
Above- 100,000 „	0	0	0	0	0	0	0	0	0	0	0	0	0	0	10	12
Presumptive Coliform Test Positive	0	0	11	30	0	0	11	23	0	0	0	0	8	11	18	18

B.T. =Brass tinned

B.U.T. =Brass untinned

No. of trials with 1 lb. bottles .. 30

„ „ 2 lb. „ .. 23

„ „ B.T. cans .. 18

„ „ B.U.T. cans .. 18

The results with brass tinned and untinned cans also show that there is practically no difference between the two treatments, i. e. washing soda and Bentonite and these two are much superior to the indigenous method. The bacterial counts of the cans cleaned with washing soda range from 300 to 2,000, the average count being 1,094 with brass tinned cans and 1,161 with untinned cans. The counts of the cans cleaned with Bentonite vary from 100 to 2,000, the average count being 1,138, with brass tinned cans and 1,166 with untinned cans. The cans cleaned by the indigenous method show counts ranging from 700 to 14,000, the average count being 4,666 and 6,083 with tinned and untinned cans respectively. The counts of the control cans vary from 6,200 to 380,000, the average count being 130,122, with tinned cans and 1,55,472 with untinned cans. Coliform is absent in all the trials with washing soda and Bentonite. In the case of the indigenous method however coliform is positive in 8 and 11 trials with tinned and untinned cans respectively. All the controls show positive test for coliform.

The primary need for clean and sterile utensils for the safe handling of milk has already been emphasized. The results of the present investigation show that Bentonite in its cleansing efficiency stands on a par with washing soda when used under conditions described above. The indigenous method although better than the controls is inferior to either washing soda or Bentonite, the reason being that the mixture of mud and cow-dung cake ash, used for cleaning, is initially laden with a high number of bacteria and it is not possible to destroy a majority of these organisms, especially the spore formers, by exposing the utensils only to sunlight. The results also indicate that for cleaning milk utensils washing soda may successfully be replaced by Bentonite which is cheaper than the former. The other advantages of Bentonite observed during the course of the experiment are that it has no caustic effect on the hands of the worker, no abrasive action on metals and it is not hygroscopic. The utensils cleaned with Bentonite also gave as clean and polished an appearance as those with washing soda. Again since it is in a powder form it can very easily be handled.

SUMMARY AND CONCLUSIONS

They are as follows:

(a) The comparative efficiency of washing soda, Bentonite and indigenous, method in sanitising milk utensils has been studied.

(b) There is practically no difference in the bacterial counts of the bottles and cans cleaned with washing soda and Bentonite.

(c) The indigenous method although much better than the controls is inferior to washing soda and Bentonite in its cleansing efficiency (as the plate counts show).

(d) Bentonite is as good as washing soda in its cleansing efficiency and may, therefore, be successfully used in place of washing soda, being cheaper than the latter, for cleaning milk utensils.

(e) Bentonite has several other advantages over washing soda such as:—
  (i) freedom from caustic effect on the hands of the worker,
  (ii) no abrasive action on metals,
  (iii) non-hygroscopic in nature, and

(f) Bentonite is in a powder form and can be easily handled.

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SEX RECOGNITION IN INDIAN CARP *LABEO ROHITA* (HAMILTON) AND *CIRRHINA MRIGALA* (HAMILTON)

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In order to determine sex from the external features of Indian carp, Mookerjee [1938] made a study of 100 specimens of *Labeo rohita* and 36 specimens of *Cirrhina mrigala* from local markets in Calcutta and obtained the following results:

Labeo rohita

(1) the pectoral fin is either greater than or equal to the anal fin in the male,
(2) the pectoral fin is less than the anal fin in female,
(3) the area around the anal opening of breeding individuals appear reddish and swollen in the female but not in the male.

Cirrhina mrigala:

(1) the pectoral fin is greater than the anal one in the male,
(2) the pectoral fin is equal to the anal one in the female.

These tests when used as a means of differentiating the sexually mature male and the female of these two species in the Punjab gave somewhat different results from those obtained by Mookerjee.

Ninety-six *Labeo rohita* and 110 *Cirrhina mrigala* were studied at Chhenawan and Lyallpur fish farms. Their sexes and weights were noted and measurements in mm. of their total length and length of the head and of all the fins and girth were recorded. The length of the fin was measured from the commencement of its base to its farthest point along its longest ray.

(i) *Pectoral and anal fins.* In *Labeo rohita*, out of 96 specimens studied, 39 were males and 57 females. In all the males the pectoral fins were greater than the anal. Among the females, 11 specimens had their pectoral fins shorter than the anal, in 5 they were equal to the anal and in 41 greater than the anal. In 24 of these 41 specimens, the difference between the lengths of the pectoral and anal fins was 5 mm. or more, and the measurements of these specimens are given in Table I (iii) (Females).

TABLE I

Comparison of pectoral and anal fins in male and female Labeo rohita (*Hamilton*)

Male					Female				
Length of body	Length of head	Length of pectorals	Length of anals	Weight	Length of body	Length of head	Length of pectorals	Length of anals	Weight
mm.	mm.	mm.	mm.	srs. ch.	mm.	mm.	mm.	mm.	srs. ch.
(i) *Pectoral fins greater than anal fins*					(i) *Pectoral fins less than anal fins*				
250	55	41	36	0— 4	305	72	45	48	0— 5
259	54	42	35	0— 4	610	110	94	95	2—12
306	56	58	46	0— 5	620	120	95	100	3— 0
312	71	63	44	0— 8	625	110	89	90	3— 2
320	72	62	48	0— 6	655	123	96	99	3—12
320	66	59	44	0— 8	665	120	101	102	4— 0
325	65	65	48	0— 6	685	120	91	98	4—12
325	70	63	47	0— 6	693	122	103	104	4—12
327	70	56	50	0— 7	720	125	110	112	6—13
330	68	65	53	0— 7	780	135	105	115	7— 8
335	77	77	56	0—10	781	135	109	111	6— 4
336	65	61	55	0— 7	(ii) *Pectoral fins equal to anal fins*				
375	78	76	59	0— 8	328	69	52	52	0— 7
398	86	82	64	0—13	337	75	57	57	0— 8
580	100	95	86	2—12	554	95	86	86	1—13
585	110	110	85	2 —8	625	115	92	92	3— 2
587	107	99	88	2 —8	654	114	106	106	3— 9
592	110	110	90	2—11	(iii) *Pectoral fins greater than anal fins*				
592	105	101	89	2— 8	300	65	50	44	0— 5
597	115	105	94	2—10	312	61	50	43	0— 6
602	115	101	95	22— 1	318	74	55	50	0— 7
610	115	103	94	2— 8	324	56	49	42	0— 8
614	110	110	91	3— 0	345	71	60	55	1— 8
620	116	113	100	3— 2	460	100	80	67	1— 6
624	116	104	94	3— 2	470	96	87	81	0— 5

TABLE I (*contd.*)

Male					Female				
Lengtd of body	Length of head	Length of pectorals	Length of anals	Weight	Length of body	Length of head	Length of pectorals	Length of anals	Weight
mm.	mm.	mm.	mm.	Srs. Ch.	mm.	mm.	mm.	mm.	sr. ch.
					(iii) *Pectrol fins greater than anal fins* (contd.)				
625	114	105	85	3— 2	511	111	89	80	1—15
630	108	103	86	3— 6	605	105	92	83	3— 5
640	123	115	102	3— 4	614	120	98	92	3— 3
640	120	109	95	3— 2	616	110	101	92	3— 3
656	115	110	101	3— 8	622	119	105	95	[illegible]— 4
656	125	105	98	4— 0	644	130	102	96	4— 6
700	130	125	105	4— 0	644	125	102	92	3— 8
706	130	128	101	4— 5	645	124	99	94	3—12
733	139	106	105	5— 2	646	120	105	96	3—10
737	130	130	115	5— 0	655	120	102	95	4— 5
					655	115	109	92	4— 6
					665	125	100	90	4—11
					666	115	107	96	4— 4
					672	130	102	94	4— 8
					672	124	100	93	4— 7
					700	128	118	102	5— 4
					750	140	103	98	6— 3

In *Cirrhina mrigala* of 110 specimens studied, 33 were males and 77 were females. In all the males, the pectoral fins were greater than the anal. In the females, only three specimens had their pectoral fins less than the anals; in two, they were equal, while in 72 the pectorals were greater than the anals. In 46 out of these 72 specimens, the difference between the lengths of the pectoral and anal fins ranged from 1 mm. to 9 mm. while in 26 the difference was 10 mm. to 23 mm. and the measurements of these specimens in which the pectorals were decidedly greater than the anal are given in Table II (iii) (Females).

TABLE II

Comparison of pectoral and anal fins in male Cirrhina mrigala (*Hamilton*)

Male					Female				
Length of body	Length of head	Length of pectorals	Length of anals	Weight	Length of body	Length of head	Length of pectorals	Length of anals	Weight
mm.	mm.	mm.	mm.	srs. chk.	mm.	mm.	mm.	mm.	srs. chk.
Pectoral fins greater than anal fins					(i) *Pectoral fins less than anal fins*				
225	42	41	33	0—2	645	109	74	83	2—13
269	49	55	39	0—2½	670	112	89	90	3—0
271	46	55	54	0—2	680	110	80	87	3—0
278	49	51	44	0—2	(ii) *Pectoral fins equal to the anal fins*				
280	52	50	39	0—3	423	76	65	65	0—10
295	51	52	43	0—3	649	110	85	85	2—10
309	67	73	56	0—7	(iii) *Pectoral fins greater than anal fins*				
364	61	70	53	0—6	290	52	46	35	0—4
373	63	73	55	0—6	371	71	63	53	0—7
380	65	60	55	0—10	570	93	88	78	1—14
391	69	73	64	0—8	608	101	88	65	2—6
406	75	78	68	0—10	622	101	80	69	2—4
407	70	72	57	0—8	625	105	95	84	2—8
505	90	82	63	1—6	635	104	100	84	2—8
574	95	96	74	1—9	642	113	97	87	2—14
613	103	100	75	2—4	645	107	101	82	2—8
619	105	95	91	2—4	655	100	95	81	2—10
620	105	100	79	2—4	664	105	90	78	2—14
633	110	110	85	2—8	669	115	100	90	3—0
635	105	94	83	2—10	670	110	97	80	3—0
640	105	93	80	2—9	688	115	107	97	3—5
640	110	91	89	2—10	696	110	94	80	3—3
648	105	101	82	2—12	700	125	105	92	4—0
650	110	110	80	3—1	702	125	102	92	3—8
655	115	99	80	3—2	712	105	112	99	3—8
655	113	99	89	2—13	718	120	109	97	3—14
656	110	121	92	2—6	722	130	107	90	3—8
668	110	105	80	2—12	724	125	103	85	3—10
693	110	130	84	2—13	725	125	109	90	4—8
694	115	116	82	3—1	727	130	105	93	4—5
700	110	100	80	3—6	735	130	98	86	3—[illegible]
714	120	108	83	3—8	742	131	95	81	4—1
					764	130	100	86	4—12

(ii) *Pectoral fins in male and female fish*: During the study it was observed that for same length of fish in *Labeo rohita* the pectoral fins of the male are better developed than those in the female. Similarly *Cirrhina mrigala*, with one or two exceptions, the same rule applied (Table III). Such special development of pectoral fins in the males of Crypinidae has been attributed by some writers [Cunningham, 1900] to special exertions of the organs during the breeding season. In the Indian carp, too, during breeding season the male is always far more active than the female which, due to heavily ladden ovaries, is always sluggish in its movements on such occasions.

TABLE III

Comparison of pectoral fins of male and female of Labeo rohita (*Hamilton*) *and of* Cirrhina mrigala (*Hamilton*) *for same length of fish*

Labe orohita				*Cirrhina mrigala*			
Male		Female		Male		Female	
Length of body	Lengte of pectorals	Length of body	Length of pectorals	Length of body	Length of pectorals	Length of body	Length of pectorals
mm.	mm.	mm.	mm.	mm.	mm.	mm.	mm.
306	58	305	45	271	55	270	47
312	63	312	50	278	51	278	45
320	62 59	320	54	280	50	279	44
325	65 63	324	49	373	73	374	60
327	59	327	54	620	100	622	82
336	61	336	59	633	110	632	97
610	103	610	409	635	91	635	100
614	110	614	98	648	101	646	87
620	113	620	95	650	110	650	89
625	105	625	89	655	99	655	95
640	115 109 122	640	99	668	105	660	100
654	118	654	86	694	116	695	90
655	121 110	655	96 109 102	700	100	700	105
700	125		118				

These observations, therefore, show that:

(1) Both in *Labeo rohita* and *Cirrhina mrigala*:

(i) when the pectoral fin is *less* than or *equal* to the anal the specimen is always a female; but a female may also have pectoral fins greater than the anals;

(ii) when the pectoral fins are *greater* than the anal, the specimen may be a male or a female, but in the male the pectoral fins are always greater than the anals;

(2) In *Labeo rohita* in the females, the pectoral fins though they may be greater than the anals, are always less developed than the pectorals of the males for the same length of fish. The same rule, with a few exceptions, applies to *Cirrhina mrigala* too.

Thus, given the same lengths of fish, it is easy to distinguish male and female in both these species, but in other cases it is rather difficult to do so, except in the breeding season when the females have bulging bellies due to the presence of ova in the ovaries and can easily be recognised.

The results obtained by Mookerjee [1938 1 and 2] are, therefore, not of universal application and it would be interesting to know if the two species of carp namely *Labeo rohita* and *Cirrhina mrigala* show similar variations with regard to the pectoral and anal fins in other parts of India as those observed in the Punjab.

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STREPTOCOCCI FROM EQUINE STRANGLES IN INDIA

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THE demand for the preparation of a strangles vaccine for experimental use in the field led us to examine streptococci from cases of strangles with a view to isolate virulent strains of *Streptococcus equi*. It was remarkable that contrary to expectation out of eleven strains of streptococci isolated from strangles abscesses, only one conformed to the description of *Str. equi*.

Schutz [1887] discovered the streptococcus of strangles but Sand and Jensen [1888] gave an adequate description, and designated the organism, *Str. equi*. This, however, was often confused with other closely-related streptococci from equines. Evans [1936] has referred to the common but erroneous practice of calling any streptococcus recovered from a disease in horses diagnosed as strangles, '*Streptococcus equi*'. This was especially the case when streptococci were classified by Holman's method. When Ogura [1929] and more particularly Edwards [1933] showed the value of trehalose and sorbitol as additional reagents, substrains could be more clearly distinguished. The studies of Bazely and Battle [1940] on haemolytic streptococci from equine infections have thrown fresh light on the subject. Besides

confirming the utility of lactose, trehalose and sorbitol as differential substances, they have shown that colony characteristics and slide agglutination tests are useful adjuncts for type differentiation. Five types of equine streptococci were found by them and the distribution of these types in various infections was indicated.

Material and methods

Of the 39 strains studied 11 were received from Major W.P.S. Edwards, R. A. V. C., who had isolated them from strangles abscesses in India. Six strains were isolated by us from material obtained by Srinivasan and Choudhury (article in press) at Mukteswar, viz. from deep swabs from the nostrils and from pus of fresh submaxillary abscesses. Two strains were isolated by Mr Shirlaw from orchitis in donkeys. Ten strains, representing types 1, 2, 3, and 4 of Bazeley and Battle [1940], were received in a dried state from Mr Bazeley of the Commonwealth Serum Laboratories, Australia. They had been isolated from fresh or previously opened strangles abscesses and from respiratory catarrh. Ten were our own laboratory strains from cases of strangles. A few of the strains mentioned above were from sources other than strangles but they were included in order to determine their type.

With all materials purity tests were first made on blood agar and representative colonies identified by biochemical tests. Stock cultures were kept on blood agar slants under paraffin. Haemolysis tests were made on 5 per cent sheep-blood agar, observations being made before and after refrigeration. Fermentation tests were made in peptone water containing 1 per cent horse serum and Andrade indicator, 1 per cent of the 'Sugar' being added from an autoclaved solution. Incubation was for seven days and seeding was heavy from an actively growing culture. The final *p*H in 1 per cent glucose broth [Avery and Cullen, 1919] was made after three days. The methylene blue reduction test was made much according to the technique of Edwards [1933], the medium being beef infusion containing 10 per cent casein digest, 1 per cent peptone and medicinal methylene blue to give final concentration of 1 : 100,000. The medium was seeded from an actively growing culture and examined after 24 hours at 37° C. Growth in bile salt was tested according to Belenky and Popowa [1929], viz. on blood agar plates containing 10 and 40 per cent sodium taurocholate. Hydrolysis of sodium hippurate was tested by the method of Ayers and Rupp [1922].

Serological. Group differentiation by precipitation tests was made according to Lancefield [1928] and type differentiation by slide agglutination tests according to Bazeley and Battle (*loc. cit.*) Sera were from rabbits which had received at five day intervals eight injections of carefully heat-killed young streptococcus culture, as advised by Bazeley and Battle.

Suspensions for agglutination tests were made as follows: Overnight cultures in tryptic digest broth, containing varying quantities of horse serum up to 5 per cent, were centrifuged and the deposit suspended in saline with 1 : 10,000 merthiolate. Sera, unabsorbed and absorbed with heterologous strains, were diluted to give specific agglutination in 20 seconds. Equal-sized drops of serum dilution and suspension were placed side by side within 1 in. squares on glass plates, run together, rocked and the time for agglutination noted.

Results

All strains belonged to Lancefield's group C, were β-haemolytic, and not heat-resistant (60°C. 30 min.). The classification adopted was that used by Bazeley and Battle (*loc. cit*). Differentiation was mainly by biochemical tests, for in slide agglutination tests, cross reactions were obtained between the types, even with the use of absorbed sero and sera diluted to end titre. Most new strains could be assigned to one or other of Bazeley's biochemical typs 1 2 and 4 (Table I). Type 1 strains (Str *equi*) differ from others by their inability

to ferment mannitol, lactose, trehalose and sorbitol. Type 2 ferment lactose and sorbitol; type 3 only sorbitol but not lactose; and type 4 are trehalose fermenters. Type 4 can also be differentiated from types 1, 2 and 3 by the ability of the former to reduce methylene blue and to grow in the presence of bile salts. Differences in colony structure [Bazeley and Battle, 1940] also proved helpful. Colonies of type 1 strains (*Str. equi*) were most distinctive, surface colonies having a zone of clear haemolysis with a very narrow peripheral zone of partial haemolysis. Colonies were mucoid, transparent and homogeneous, drawing to a thread when touched by a loop. Adjacent colonies had a tendency to coalesce. The haemolytic zone in this type was biggest in relation to the size of the colony. Differences between colonies of types 1, 2 and 4 were not sufficiently distinctive to enable one to place them definitely in their respective types without reference to biochemical tests. As a rule, colonies of streptococci of types 2 and 3 had a larger haemolytic zone than those of type 4. Type 4 colonies were granular and opaque, had a central opaque nucleus and portions of the colony were buried in the medium. Type 3 colonies were relatively transparent, as also were the margins of those of type 2.

Table I

Streptococci from strangles in India

Strain	Biochemical type	Disease	Reaction in						Final *p*H	Methylene blue	Growth in presence of bile salts	
			Mannitol	Salicin	Lactose	Trehalose	Sorbitol	Sod. hippurate			10%	40 %
1		Resp. catarrh ..	—	+	—	—	—	—	4·8	—	—	—
2	1	Fresh submax. abscess ..	—	+	—	—	—	—	4·8	—	—	—
3—4		Open submax. abscess ..	—	+	+	—	+	—	4·8	—	—	—
5		Rep. catarrh ..	—	+	+	—	+	—	4·8	—	—	—
6	2	Sinusitis ..	—	+	+	—	+	—	5·4	—	—	—
7—9		Fresh? Submax. abscess ..	—	+	+	—	+	—	(1) 4·8, (2) 4·6	—	—	—
10—11		Orchitis ..	—	+	+	—	+	—	4·6	—	—	—
12	3	Resp. catarrh ..	—	+	—	—	+	—	4·5	—	—	—
13—15		Fresh sub-max. abscess ..	—	+	—	+	—	—	(1) 4·6, (2) 5·0	+	+	—
16		Resp. catarrh ..	—	+	—	+	—	—	4·8	+	+	—
17—22	4	Fresh? submax. abscess ..	—	+	—	+	—	—	(1) 4·7, (5) 4·8	+	+	—
23—25		Strangles-Respiratory symptoms only-Nasal swabs-same outbreak as Nos. 26 to 28	—	+	—	+	—	—	4·8	+	+	—
26—29		Fresh submax. abscess ..	—	+	—	+	—	—	(1) 4·8, (3) 5·0	+	+	—
30		Fresh submax. abscess ..	+	+	+	+	—	—	4·0	+	+	—
31	2*	„ „ „ ..	+	+	—	+	+	—	4·0	+	+	—
32—36		Fresh submax. abscess ..	+	+	—	+	+	—	(2) 4·0, (3) 4·2	+	+	+
37	1*	Fresh submax. abscess ..	—	+	+	—	—	—	4·2	—	+	—
38		Kidney abscess ..	+	+	—	+	+	—	4·0	+	+	+
39		Hock joint ..	—	—	—	—	—	—	5·0	+	+	—

Strains 1 to 6 and 12 to 16 are of Australion origin and were received from Mr Bazeley.
Variants of type 2 or type 1 strains, as indicated.

Of 11 strans isolated from strangles abscesses by Edwards, one was type 1, i.e. *Str. equi*, two were type 2, six were type 4 and the remaining two were mannite fermenters falling outside the classification of Bazeley and Battle. The six strains isolated from nasal discharge and freshly opened submaxillary abscesses of cases of strangles in this Institute, belonged to type 4. Of the ten strains in stock and isolated between 1923 and 1931, 8 had been isolated from strangles pus; of these, one was type 2, one type 4 and the other six fell outside Bazeley and Battle's classification. Of these six strains, five somewhat resembled variants of type 2 strains of Ochi and Hirao [1940], as also was the case with Edwards' mannite-fermenting strains. The sixth resembled the first variant from type 1 strains of the same workers.

Discussion

Bazeley and Battle found that strains isolated from freshly opened strangles abscesses were exclusively *Str. equi* and this conforms with the findings of most other workers. But it was remarkable that all the strains isolated from freshly opened sub-maxillary abscesses in the outbreak, clinically diagnosed as strangles at this Institute, were type 4. Six of 11 recently isolated strains from cases of strangles forwarded by Edwards were also type 4; only one was *Str. equi*, two were type 2 and two others were probably variants of type 2. Haddow and Iyer [1939], who examined 31 strains from cases of strangles, found 9 only which conformed to the description of *Str. equi*.

The paucity of true *Str. equi* (i. e. non-lactose fermenter), the finding of type 4 in one outbreak to the exclusion of all other types, and the general preponderance of types other than *Str. equi* among strains isolated from cases of strangles in India call for some comment. If the strains had been isolated from abscesses opened several days previously, the preponderance of types 4 and 2 might be explained on the analogy of the findings of Bazeley and Battle [1940]. But, though we have no data on this point regarding Edwards' strains, we know that the Institute strains, which were all exclusively type 4, were obtaind from *freshly-opened* abscesses.

We have been accustomed so far to consider 'strangles' as a specific disease caused by *Str. equi*. In view of the findings reported in this article, and in accordance with the definition put forward by Minett [1944] 'strangles' may have to be accepted as a term used in a clinical sense to a disease of horses and mules involving the upper respiratory tract, frequently accompanied by suppuration of glands about the throat and caused by haemolytic streptococci belonging to Lancefield's serological group C though not necessarily of the same biochemical and serological type.

Admittedly, the number of strains used in this study is small, and it may not be possible to draw general conclusions. But these findings call for the detailed study of a sufficiently large number of Indian strains from animals whose clinical history is known.

Summary

Thirty-nine strains of streptococci from equine sources have been classified according to the method of Bazeley and Battle [1940]. Seventeen of these were recently isolated from cases of strangles in India. Contrary to expectation, only one of the seventeen strains was *Str. equi*., while 12 belonged to type 4 of the authors named. Six strains from one outbreak belonged to type 4, two others were type 2 and two were possibly variants of type 2.

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OBSERVATIONS ON THE LIFE-HISTORY OF *HAEMAPHYSALIS BISPINOSA* NEUMANN*

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OBSERVATIONS on the life-history and Bionomics of this parasite which is the commonest one in Newzealand have so far been made by Miller [1922] and Myers [1923].

These workers have made their observations under field conditions without keeping any record, whatsoever, of the atmospheric conditions; and again their observations are only in round and approximate figures. For example the parasitic periods in all the three stages namely larvae, nymphs and adults, are recorded as extending over one week, while the non-parasitic periods in both the larval as well as the nymphal stages are observed as three weeks.

In the present communication, however, it was proposed to study the life-history in fuller details but unfortunately the whole consignment procured for this purpose from the Veterinary Assistant Surgeon, Sirsi, N. Kanara Dist. (Bombay Province), was found to be parasitized by the Encyrtid, *Hunterellus hookeri* (order Hymenoptera) and only a few adults were available for the purpose of this study. The observations were started in May 1938, on the receipt of the consignment and bullocks and goats were used for feeding the ticks. In their non-parasitic stages, the ticks were kept at a temperature of 22°C.

I *Oviposition.* Seven gravid females were available. The duration of oviposition ranged from 7 to 15 days, while the period of survival of the female after oviposition ceased was three to six days. The female lays from 784 to 1849 eggs (average 1333·4).

II *Egg stage.* Only some 600 to 700 larvae emerged from the lot of about 9,000 eggs. The duration of the egg-stage as calculated from the date of commencement of oviposition to the date of emergence of the first larva was on an average 30 days.

III *Larval stage.* Recently emerged larvae, numbering about 675, divided into several batches, were put on goats and hill-bulls for feeding. Percentage recoveries of engorged larvae were as follows:

	DAYS						
	4	5	6	7	8	9	10
Goat ..	1·5	4	54	29	0·5	..	..
Bull ..	5	22·5	48·5	8	1	..	1

* Paper read at the Indian Science Congress, Baroda, January 1942

In the batches from the goat nymphs emerged after 17 to 24 days, most on 20 and 21 days, while in those from the hill bull the nymphs emerged after 18 to 25 days.

IV *Nymphal stage.* On 25-8-38 about 100 nymphs were placed on a goat and an equal number on a hill bull. They fed on the goat for 6 to 12 days, most leaving the host after seven and eight days. Nearly half the number placed on the bull were lost accidentally and of the rest most left the host after eight days, but one individual remained attached for 15 days. A total of 153 adults from both groups emerged after 28 to 34 days, comprising of 63 males and 90 females.

V *Adult stage.* Observations on the parasitic period of the adult stage were not carried out in continuity of the above experiments, but were made at a later date only on goats. About 14 adults were observed which dropped off as follows: two on day eight, one on day nine, four on day 10 and seven on day 12.

SUMMARY

Haemaphysalis bispinosa was reared easily on goats and hill-bulls. The oviposition period was 7 to 15 days and the period of survival of the female after oviposition ceased was three to six days. The duration of egg-stage was on an average 30 days. Larvae were fully engorged in 4 to 10 days. Nymphs remained attached for 6 to 12 days, in exceptional cases for 15 days. The parasitic period of the adult was from 8 to 12 days. The larval and nymphal periods (non-parasitic), counting from the date of engorgement, were 17 to 25 and 28 to 34 days, respectively at 22°C. Of 153 adults, 63 were males and 90 females.

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A NOTE ON PULLORUM DISEASE SURVEY IN INDIA

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(Received for publication on 30 August 1944)

As is well-known, bacillary white diarrhoea or more correctly 'pullorum disease' has assumed serious proportions in many countries, the increase in incidence running parallel with the progress in artificial incubation and baby chick traffic. Cooper and Naik [1931], from an outbreak in the United Provinces, isolated a microorganism which they typed as *S. gallinarum.* (*S. pullorum* is now classed as a variant of *S. gallinarum.*) Naidu [1938] reported its occurrence in large number of fowls from Mysore state.

As the poultry industry is rapidly developing in India and as a few farms have actually started the production and supply of artificially-hatched baby chicks, an extensive survey of the country was undertaken to find out the incidence of this disease. State and Provincial Poultry Disease Officers also cooperated by undertaking similar surveys within their areas.

EXPERIMENTAL

For the detection of pullorum-infected fowls the rapid whole-blood stained-antigen test, as described by Schaffer, Macdonld. Hall and Bunyea [1931] and used by English and American workers, was employed with slight modifications. Three authentic strains of *S. pullorum*, from the Veterinary Laboratory of the Ministry of Agriculture in England, were incorporated in the antigen prepared for the test. A drop of blood from the wing vein or the snipped comb was mixed on a glass plate with an equal quantity of the standardized antigen (a thick, stained and killed saline suspension of *S. pullorum*).

Clumping (agglutination) of bacteria occurred in a minute or two if the blood was from an infected fowl, otherwise the mixture remained uniformly homogeneous. This test is rather delicate, but is particularly useful under field conditions.

The birds examined were all mature and most of them from breeding stock. They belonged to 23 poultry farms, spread over U. P., Bihar, Orissa, Mysore and Punjab. These farms are all organized and run on progressive lines (artificial incubation, etc.) and should thus constitute the probable foci of infection if any were present. In all, 3798 birds were tested but none of them was found to be infected. In addition, the Poultry Disease Officers of Mysore, C. P., Madras, Bengal and Assam examined a further number of 1219 birds. Out of these, only 11 birds from Mysore (Hebbal Farm) gave doubtful reactions, the rest reacting negatively. Bunyea and Macdonald [1941] Holm, William, Callahan and Halversen [1940] and Johnson and Pollard [1940] described certain conditions leading to false or non-specific reactions with this test. Materials from the suspicious reactors were therefore submitted to more detailed laboratory examination, but all proved negative for *S. pullorum* infection.

These results suggest that pullorum disease is of no great significance at present to the poultry industry in this country. This apparent freedom is probably due to the absence of commercial hatcheries engaged in baby chick traffic. In view of the fact that the disease has been responsible for enormous losses in other parts of the world, it is essential that every possible effort should be made to locate and stamp out any possible source of infection, so as to prevent its spread throughout the country. Careful vigilance by the livestock departments with periodic surveys of areas under their jurisdiction and the education of all concerned in the diagnosis and control of the disease should therefore form an integral part of their poultry improvement plans. It is also suggested that all imported birds should be tested prior to their inclusion in the stock, so as to prevent the introduction of the disease into the country.

SUMMARY

A total of 5017 adult fowls were tested for pullorum disease by the quick agglutination method with completely negative results, save in the case of 11 birds which gave suspicious reactions. A more detailed examination of these birds did not show pullorum infection. The birds included in this survey were mostly from organized poultry farms, situated in U.P., Bihar, Orissa, Punjab, Mysore, C.P., Madras, Bengal and Assam. The survey was therefore well-distributed and representative.

ACKNOWLEDGEMENTS

The authors express appreciation for the cooperation of Assistant Disease Investigation Officers (Poultry) in this survey.

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A CASE REPORT OF BOVINE ENCEPHALOMYELITIS AT THE BENGAL VETERINARY COLLEGE HOSPITAL

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(Received for publication on 6 March 1944)

The object of this paper is to place on record the occurrence of Encephalomyelitis in a cow in the Bengal Veterinary College Hospital during the month of November, 1943.

Introduction

Borna disease (Syn:—Enzootic meningo-encephalomyelitis) occurs primarily in horses but cattle, sheep, deer, etc. also become infected. Nicolau and Galloway [1927] had shown that a variety of domesticated animals is susceptible to a specific encephalomyelitis caused by the virus of Borna disease. This disease has a wide distribution in Europe, particularly in Germany, and also in America. A disease of young cattle described by McNutt [1940] as primarily an infectious encephalitis and meningitis, having a close resemblance to *Listerella* infection, was later found to be due to a virus—the probable etiology of bovine encephalomyelitis. An outbreak of bovine encephalomyelitis, caused in all probability by an ultra-visible virus, has been recorded from the Imperial Veterinary Research Institute, Izatnagar [Hassan and Idnani, 1943]. The present paper deals with what appears to be the similar affection in a six-year old grey Nagore cow, which was admitted in the Bengal Veterinary College Hospital on 19-11-'43 for invistigation, diagnosis and treatment.

Symptomatology

The onset of the disease was sudden. Restlessness, stamping of the feet, bellowing, staggering, attacking the surrounding objects switching of the tail and frequent attempts at micturition were the early symptoms observed. Temperature was elevated only slightly above the normal, bowels were constipated, urine was scanty and rumination was stopped. Hyperesthesia in the region of face, neck and along the vertebral column was pronounced and reflex irritability was increased. Slight external stimuli were sufficient to cause general cramp. The period of excitement lasted for about 24 hours and was followed by progressive depression. In co-ordination with these loss of power in the hind limbs became manifest. Later, paralysis supervened and the animal collapsed. Twitching of the superficial muscles, specially of the face and neck, with spasms of the extremities, specially of the hind legs, were marked. There was difficulty in swallowing from the very outset and saliva flowed out of the mouth. The neck became stiff and was bent backwards. Pupils were dilated and the reaction to light was entirely absent. Respiration during the period of excitement was accelerated, but afterwards retarded and became superficial. Temperature came down to normal on the termination of excitement and then became subnormal towards the end. Hyperesthesia and reflex irritability, which were increased at the commencement, passed off as the disease progressed. Repeated straining resulting in prolapse of the rectum and vagina was a marked feature of the disease. Towards the end, the animal passed on to coma and complete paralysis and died on 29-11-43, i.e. after suffering for ten days.

Post-mortem findings and laboratory diagnosis

The only organs which showed changes of specific nature were the brain, the spinal cord and the meninges. These organs showed numerous pinpoint

haemorrhages and the cerebrospinal fluid both in the meningeal spaces and the ventricles of the brain appeared to be clear and increased in amount. The gastro-intestinal tract showed inflammatory changes. Microscopical examination of smears from different organs of the body yielded negative result, and an attempt at cultivating bacteria from the brain proved futile. Smears from hippocampus was examined and found to be negative for Negri bodies. Pieces of brain and spinal cord fixed in 75 per cent alcohol were sent to the Imperial Veterinary Research Institute, Izatnagar, for histo-pathological examination and the following report was received: 'The specimen from the cow revealed extensive degeneration of the nerve cells and very marked perivascular cuffing on microscopical examination. These changes are indicative of encephalomyelitis'.

The above findings confirmed the result of the following transmission experiments conducted in the College Laboratory.

Transmission experiments

Small pieces from representative areas of brain and spinal cord were triturated in normal saline solution with aseptic precautions and an uniform emulsion thus prepared consitituted the inoculum for the following transmission experiments.

G. P. 1. Inoculated subdurally with 0.1 c.c. inoculum obtained from the cow, developed paralytic symptoms on the 17th day, died on the 18th day, period of incubation 17 days; P.M.—Encephalomyelitis.

G. P. 2. Serially inoculated subdurally with 0.1 c.c. saline emulsion obtained from G. P. 1; paralysis on the 15th day, died on the 16th day, period of incubation 15 days, P.M.—Encephalomyelitis.

Bull-calf 1. Intranasal instillation with saline emulsion of brain and spinal cord of G. P. 1, developed inco-ordination on the 22nd day followed by progressive paralysis and death on the 25th day, period of incubation—22 days, P. M.—Encephalomyelitis.

G. P. s 3 and 4. Inoculated subdurally with 0.1 c.c. saline emulsion of brain and spinal cord of bull-calf 1, developed paralysis on the 19th day, died on the 21st day, period of incubaton—19 days, P. M.—Encephalomyelitis.

Note: Attempts at cultivating bacteria from the central nervous system of the test animals proved negative.

Acknowledgement

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CANINE LEPTOSPIROSIS IN CALCUTTA

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(Received for publication on 10 July 1944)

(With Plate II)

*Leptospira icterohaemorrhagiae** was discovered by Inada and Ido [1915] who observed the organism in the blood and tissues of patients suffering from Weil's disease. A year later, Uhlenhuth and Fromme described a case of infective jaundice in the dog with *Leptospirae* in the liver and suggested the possibility of a connection between the disease in the dog and Weil's disease of man. Since then leptospiral disease in dogs has been recorded by various workers in different parts of the world. In localities where canine leptospirosis exists, two types of the disease are usually noted:—(1) A disease identical with Weil's disease in man and accompanied by jaundice, the causal organism being *L. icterohaemorrhagiae.* (2) Another type which is more frequent, *L. canicola* being the infective agent. The disease caused by *L. icterohaemorrhagiae* usually occurs in young dogs, and runs an acute course, and jaundice is a prominent feature, whereas the disease caused by *L. canicola* runs a more chronic course and is characterized by uraemic symptoms, and in most cases jaundice is absent. Moreover, other types of *Leptospirae*, especially *L. hebdomadis* of Akiyami A type, have been known to occur in the dog [Fletcher, 1927].

In India, Ayyar [1932] described an outbreak of leptospiral jaundice among the Madras hounds. Of the 12 dogs examined *post mortem* by this worker, the liver and kidney substance of only one animal showed forms indistinguishable from *Leptospira* and out of 31 guinea-pigs inoculated with different materials from these dogs only on two occasions were *Leptospirae* definitely revealed in the liver. Sera of 4 dogs which survived were tested at the Medical Research Institute, Kualalumpur, and it was found that all these specimens reacted with the classical *L. icterohaemorrhagiae.* It is rather unfortunate that it was not possible to obtain a culture of the causal organism in any case, although there was ample opportunity in that quite a number of animals was involved in this outbreak.

* The organism was named *Spirochaeta icterohaemorrhagiae* by its discoverers. Later on, when its morphology was studied by Noguchi, it was found to be so distinctive that a new genus was established and the organism was renamed *Leptospira icterohaemorrhagiae.*

Present investigation

In February last Major General W.C. Paton informed the senior author that his dog was suddenly taken ill, developed jaundice and died within three or four days of the onset, which aroused the suspicion that leptospiral disease probably exists in Calcutta. It may be recalled here that the occurrence of human leptospirosis has already been reported in this city [Das Gupta & Chopra, 1937].

On 24-3-1944 (about a month or so after the death of General Paton's dog), a Dachsund pup, aged 5½ months, became acutely ill, refused all food in the morning and took a little in the afternoon only to vomit immediately after.

- 25-3-44 Passed loose stools containing blood. Tempeature 99°F.
- 26-3-44 There was frequent vomiting and the vomited matter contained blood; conjunctivae slightly icteric.
- 27-3-44 Vomiting persisted; jaundice more marked.
- 28-3-44 Developed intense jaundice and difficulty of respiration. The animal appeared almost moribund.

Dr Anthony who was treating this pup, diagnosed the case as one of leptospirosis on clinical grounds. In the light of this suggestion the following examinations were undertaken:—

Blood serum was put up for agglutination test against *L. icterohaemorrhagiae* and *L. canicola*.

The animal was sacrificed. Sections were taken from liver and were stained by haematoxylin-eosin for a histo-pathological study. Portions of the liver and kidneys were ground up and emulsified in normal saline. Several preparations of the emulsion were examined under dark-ground illumination for *Leptospira*. Four young guinea-pigs and three full-grown dogs (as young dogs were not available at the time) were inoculated intraperitoneally with this emulsion.

RESULTS

Pup.—The serum did not react with *L. icterohaemorrhagiae* but agglutinated *L. canicola* in low dilutions (upto 1:40). This reaction is apparently non-specific, inasmuch as this particular strain has been found to react with fresh human and various animal sera in low dilutions upto 1:80 [Das Gupta, 1942].

No *Leptospirae* could be seen in the liver emulsion in spite of prolonged search. Section of liver revealed necrosis and fatty degeneration (Plate II fig. 1).

Inoculated animals:—

(a) *Dogs*.—All dogs remained alive and well for three weeks.

(b) *Guineapigs*.—Out of four guinea-pigs, two developed typical signs of leptospiral infection. Of these one died on the 17th day of inoculation and on the next day the other animal was almost dying, when it was killed. The liver emulsion of this animal was found to show a considerable number of actively motile *Leptospirae*. Also sections were taken from the liver and stained by Levaditi's method; these were crammed with *Leptospirae* arranged at the periphery of the liver cells (Plate II, fig. 2). Heart blood containing a fair number of the organism was cultured on Vervoort's medium. As only scanty growth was obtained in this medium, cultivation on Fletcher's medium was tried. After repeated subcultivation in the latter medium there was a luxuriant growth which was utilized in performing agglutination tests against various anti-sera prepared in this laboratory.

Table showing agglutination reaction with anti-sera prepared against a dog strain, three human strains and one rat strain.

(The dog strain was obtained from the Lister Institute, London, and the others were isolated by the authors)

Source of the strain	Anti-sera	Results
Leptospira isolated from Dr Cox's pup	*Leptospira canicola* (dog strain)	0
	Andamans CH 31=*L grippotyphosa*	0
	Andamans CH 11	0
	L. icterohaemorrhagiae (human strain)	1:10,000
	L. icterohaemorrhagiae (rat strain)	1:10,000

COMMENTS

It will be seen from the foregoing that in spite of laborious search of the liver and kidney emulsion of the pup under dark-ground illumination it was not possible to demonstrate the *Leptospira*. Of the three dogs inoculated with the emulsion none developed the disease. Out of four guinea-pigs which received a large dose of the emulsion, only in two animals was the infection produced. If only one animal had been used for the diagnostic inoculation, as is usually done, the infection might have possibly been missed. It follows, therefore, that the isolation of *Leptospira* in dogs in some cases at least may be extremely difficult.

SUMMARY

A case of leptospiral jaundice in the dog caused by *Leptospira icterohaemorrhagiae* is described.

ACKNOWLEDGEMENTS

The writers are deeply indebted to Major General W.C. Paton, K.H.P., I.MS., for mentioning the case of his own dog to them, thus suggesting the possibility of the existence of canine leptospirosis in the city, and for his keen interest throughout the investigation. Their grateful thanks are also due to Dr J. E. Anthony and to Dr E. Cox for bringing the case under report to their notice, and to Captain C. Graham Eddy of the 1st Medical Detachment, Museum & Medical Arts Service, U. S. Army Medical Museum, for preparing the photomicrographs used in this paper.

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FATAL ENTERITIS IN GOATS DUE TO IMMATURE AMPHISTOMES, PROBABLY *COTYLOPHORON COTYLOPHORUM**

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(Received for publication on 28 April 1944)

(With Plate III)

DURING the months of February and March 1943, 19 out of a herd of 40 goats in the Madras Pinjrapole *gosala* succumbed to disease. During the day, it had been the custom to take these animals out to graze to an area round about a tank bed near the Pinjrapole, and at night to pen them in the stalls of the *gosala*.

The symptoms exhibited by the diseased animals were a general weakness with suspended feeding, great depression, inability to move about and œdema of the subcutaneous tissues, particularly of the lower jaw. The swelling in the region of the jaw appeared very predominant in the morning but was less noticeable in the evening. The goats were visibly ill for a couple of days and by about the third day they exhibited symptoms of diarrhoea. The faecal matter was dark in colour, of a thin consistency and had an offensive smell. This was followed by prostration and death in about five or six days.

Attempts to diagnose the condition during life were unsuccessful. The only feature noticed which could throw light on its etiology was severe anaemia accompanied with intense leucocytosis, particularly of the neutrophiles and eosinophiles. The faecal examination revealed a few ova of strongyles and amphistomes.

POST-MORTEM APPEARANCES

It was not possible to conduct a post-mortem examination on all the carcases. A careful autopsy on one of the carcases, however, revealed the following features:

The rumen contained a few adult and some immature forms of *Cotylophoron cotylophorum* and a very few specimens of *Fischoederius elongatus*. The abomasum was badly congested. It was noteworthy that no forms of the large stomach worm *Haemonchus contortus* were encountered. The pyloric end of the abomasum (Plate III, fig. 1) was inflamed, oedematous and had a number of petichae. A casual examination revealed several immature amphis-

*Paper presented before the Indian Science Congress in year 1944.

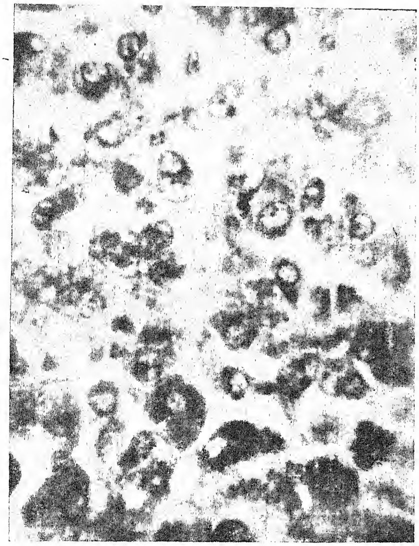

FIG. 1. Section of liver of the pup showing necrosis and fatty degeneration. Haematoxylin and eosin stain. (×1000 approx.)

FIG. 2. Section of liver of an infected guinea-pig showing a very heavy infection. Note that the *Leptospirae* are arranged at the periphery of the liver cells. Levaditi's stain. (×1000 approx.)

PLATE III

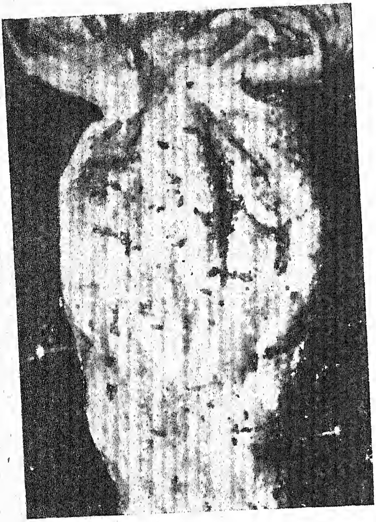

Fig. 1. Pyloric end of abomasum and the duodenum showing inflammation, thickening and oedema and studded with immature forms

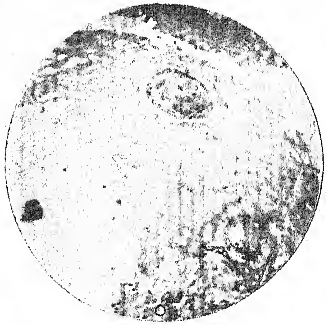

Fig. 2. Section of duodenum showing a longitudinal section of immature amphistomes under the submucosa

tomes sticking out of the inflamed mucosa. The points of attachment of the worms were marked by petichae and breaks in the mucosa. The duodenal mucosa was inflamed, thickened and oedematous. The surface was studded with small immature parasites, of a size varying from that of a mustard seed to that of a small melon seed. Acute catarrhal enteritis was present. This was most prominent in the first foot of the duodenum beyond which the lesions became gradually less intense. The walls of the large intestines showed very few calcified nodules, but in their lumen a small number of *Oesophagostomum columbianum* was found. The few strongyle ova detected in the faeces were probably of this species of worm. No flukes were met with in the gall-bladder or bile ducts, although the liver showed fatty changes.

As regards the other organs, nothing beyond a pallidity, probably caused by the loss of blood, could be detected.

HISTOPATHOLOGY

A sectional study of the duodenum (Plate III, fig. 2) revealed an acute catarrhal enteritis, with a cellular infiltration. A longitudinal section of the immature worm (Plate III, fig. 2) was seen in the submucous area and its well-developed acetabulum enclosing a piece of the mucous tissue indicated considerable irritation caused by these immature worms.

OBSERVATIONS

The mature worms obtained from the rumen were examined and mostly found to be *Cotylophoron cotylophorum* and a very few *Fischoederious elongatus*. No other helminths were met with in the rumen. The immature worms from the duodenum also appeared to be *Cotylophoron cotylophorum*, though it is obviously dangerous to attempt an identification of any worm, particularly of amphistomes, at the immature stage. These immature worms were at different stages of development. Some had developed the intestinal caeca, while in others were seen only the sexual primordia. In a few others, the genitalia could be distinctly traced, though they had not begun functioning. The mature worms in the rumen had ova in their uteri and probably a few ova found in the faeces were from these worms. That the cause of the enteritis and death was due to these immature forms of *Cotylophoron cotylophorum* is quite apparent.

The pond or tank around which the goats grazed was examined to ascertain its molluscan fauna and larval trematodes. The molluscs met with were *Indoplanorbis exustus*, *Limnea leuteola* and *Vivipara bengalensis*, of which only the *Indoplanorbis exustus* was found to discharge cercariae which resembled *Cercariae indicae* XXVI (Sewell, 1922), while the other two molluscs were free from any infestation. It is possible that these cercariae may be the larval forms of the amphistomes in question. The actual percentage of infection with the cercariae could not be determined, as the molluscs were already dying off owing to the scarcity of water in the tank on account of the hot weather. Further investigations on the determination of the cercarial fauna of this area will be undertaken at a more propitious season.

DISCUSSION

There appears to exist considerable confusion with regard to the identity of the adult amphistome of *Cercariae indicae* XXVI (Sewell, 1922). Rao and Ayyar [1932] evolved the adults of these cercariae by feeding experiments and expressed the opinion that the adults obtained were *Paramphistomum cervi*. These authors had obtained only immature forms and based their diagnosis on these. The cercariae of *Paramphistomum cervi*, according to Bennett [1936], are lacking in the evaginations from the excretory canals, which formed a prominent feature in *Cercariae indicae* XXVI. Hence, it is doubtful if the adults obtained are not of *Cotylophoron* species.

Bennett [1936] has described the cercariae of *Cotylophoron cotylophorum* in the life history studies on that trematode

and concluded that *Cercariae indicae* XXVI (Sewell, 1922) is not the larval form. His description of the excretory system of the cercariae which he has found for the adult parasite agrees with that in *Cercariae indicae* XXVI. The differences noticed by him do not seem to justify his claim that it is different. I am of opinion that Bennett [1936] was actually dealing with *Cercariae indicae* XXVI (Sewell, 1922).

Srivastava [1938] in the life history studies of *Cotylophoron cotylophorum* has mentioned the larval form of this parasite to be very similar to *Cercariae indicae* XXIX, though not identical with it. It has already been established by Rao and Ayyar [1932] and Vaidyanathan [1941] that the adults of these cercariae are *Fischoederius elongatus* belonging to a different genus altogether. Further, *Cercariae indicae* XXIX has been so far recorded only from *Limnaea leuteola* and not from *Indoplanorbis exustus*.

Pande [1935] records an acute amphistomiasis of cattle in Assam caused by immature forms of *Paramphistomum cervi*. During a personal discussion with him, he expressed the opinion that the immature forms are probably of *Cotylophoron* species and that he was not convinced that they were of *Paramphistomum cervi*.

Le Roux [1930] has dealt with two severe outbreaks of amphistomiasis due to *Cotylophoron cotylophorum* in sheep and goats.

Chatterjee [1931] in his preliminary observations on the life history studies with *Cercariae indicae* XXVI recorded that the young forms developed in his experimental goats by feeding with the above cercariae were of *Paramphistomum cervi*; however, in a later communication, the same author [1938] changed his opinion and pointed out that the cercariae in question was the larval form of *Cotylophoron cotylophorum*.

Thus there are more cases of amphistomiasis caused by immature forms of *Cotylophoron* species on record than by *Paramphistomum cervi* or any other amphistoma. There also appears to be no concensus of opinion regarding the adult of *Cercariae indicae* XXVI (Sewell, 1922).

In the present instance the mortality among the goats has been presumed to have been caused by immature forms of *Cotylophoron cotylophorum*, since the only adults seen in the rumen were of that variety and the number of *Fischoederius elongatus* encountered (about six) was negligible. In the course of the routine post-mortem examination of ruminants, the writer has found adults of *Cotylophoron* species more frequently than *Paramphistomum* species. Hence, the writer is inclined to think that the incidence of the species *Cotylophoron*, at any rate in south India may be much more than that of *Paramphistomum*, though further statistical evidence of this is necessary. It is hoped that such evidence will become available shortly.

Summary

An outbreak of amphistomiasis among goats has been described from Madras and the cause has been provisionally determined to be the immature forms of *Cotylophoron cotylophorum*.

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A STUDY OF THE VITAMIN A AND CAROTENE CONTENT OF COW'S MILK DURING ONE COMPLETE LACTATION

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The importance of vitamin A as an essential ingredient of human diet cannot be over-emphasized. A regular supply of it to the system is indispensable for the proper development and functioning of the body and as a preventive measure against susceptibility to disease. A number of workers have made quantitative studies on the daily requirements of Vitamin A (or its precursor carotene) for various types of animals, for keeping them in good health. Thus, Ward, Bechdel and Guerrant [1938] found that 12-18 microgrammes of carotene are required per lb. of body weight per day for growing dairy calves to prevent A avitaminosis. Converse and Meigs [1938] have deduced the carotene requirements of cows for normal reproduction and calving to be 80-100 mg. daily during the last months of gestation; the amount below 60 mg. per cow per day resulting in a considerable proportion of still-born calves. Kuhlman and Gallup [1941] working with lactating cows of Jersey breed arrived at the conclusion that 40-45 microgrammes of carotene per lb. of body weight (or 40-45 mg. for a 1,000 lb. animal) is about the minimum daily requirement for normal lactation. With amounts lower than 40 microgrammes, the lactation was likely to be impaired; the calves born were weak and the cows developed abnormal conditions. Similarly, it has been ascertained that about 4 mg. is the minimum daily dose for man.

The most important source of supply of vitamin A for cattle is green roughage. Seshan and Sen [1942] have determined the carotene content of a large number of green roughages ordinarily fed in this country, and the amount of fodder necessary to meet the vitamin requirements of main types of animals can easily be calculated. The problem of feeding cows, however, is somewhat different. As a milch animal, the cow requires vitamin A not only for her own system but also for secretion in milk which forms one of the important sources of vitamin A for the consumer. Studies on the relationship between the carotene content of the feed and that in the milk have been carried out by many workers. Moore [1932] has shown that in the cow carotene of the feed undergoes conversion to vitamin A and is secreted in the milk partly as carotene as such and partly as vitamin A. Kennedy and Dutcher [1922] demonstrated as early as 1922, that the vitamin-A value of milk is influenced by the vitamin-A value of the feed.

Kraus [1931], Watson [1932] and others have shown that pasture grass is rich in vitamin-A factor and produces a milk of high vitamin-A activity. Recently a more quantitative study has been made of this problem. Hogdson and co-workers [1938] have found that the average percentage of the carotene ingested which was secreted as carotene in the butter fat was 0·71 for cows receiving hay, 0·22 for cows receiving hay and silage, and 0·12 for cows receiving silage only. Similarly, Bartlett and co-workers [1938] and Seshan and Sen [1942] arrive at the same conclusion, viz. increased intake of carotene is followed by the increased carotene and vitamin-A potency of milk.

The studies of Henry, Housten and Kon [1940] on the vitamin content of the first milk or colostrum have brought out its amazing richness in vitamin A and

carotene. They found colostrum to be 35 times richer in vitamin A and 65 times richer in carotene than the later milk. From this it appeared worthwhile to study the vitamin content of milk at different periods or stages during one complete lactation in the Sahiwal breed of cows.

Experimental

Five Sahiwal cows weighing about 800 lb. each, in their fourth lactation, were employed for this purpose. This experiment was started about two months after they had calved and so colosturm could not be included in this study. During the early part of the experiment, i.e. April and May 1942, these cows were on green berseem, and then from June to November when the experiment ended with the end of the lactation, they were on green maize, and these fodders were fed *ad libitum*, in addition to a quantity of the concentrate mixture of bran and cotton seed. A composite sample of morning and evening milk was taken daily for fat determination and the weekly estimation of vitamins.

The estimation of carotene and vitamin A in milk was carried out according to the method of Willstaedt and With [1938] in which 100 c.c. of well-shaken milk is mixed with one tenth of its volume of 60 per cent KOH solution. The flask is filled with nitrogen, stoppered, and vigorously shaken, and kept aside for 48 hours with occasional shaking. After this cold saponification, 20 c.c. of alcohol is added, and the whole extracted with peroxide-free ethyl ether, extract washed with water, dried over anhydrous sodium sulphate, and the ether evaporated in an atmosphere of nitrogen. The residue is taken up in chloroform for vitamin A or in petroleum ether for carotene, and the colour compared in Lovibond Tintometer for blue and yellow values.

The data obtained are given in Table I. The first two columns show intake of green fodder (average of the previous seven days) and carotene contained in it. Columns 3,4 and 5 give the total output of butter in grammes, carotene and vitamin A in milligrammes respectively, for each of the five cows. Now, turning to the column for green fodder intake by each of the cows we find that, as these were allowed as much as they would eat, they consumed between 60 and 100 lb. of berseem daily (upto 23-5-44), and later on when maize was the only green roughage, the average daily consumption varied considerably with each cow going up to a maximum of 110 lb.

The amount of carotene intake during the berseem period varied naturally with the amount of feed ingested and was between 1520 mg. and 4950 mg. per day. During the latter period (maize period) the intake decreased considerably due to the fact that maize is not so rich in carotene as berseem, although the consumption of the former was more or less the same.

The output of carotene in milk was about 2 to 4 mg. daily, and this remained fairly constant for a considerable time practically throughout the period of lactation except in cows No. 4 and 5 where it fell to a figure round about 1 mg. during the latter part of the lactation.

The output of vitamin A, calculated from the blue units into milligrams according to the formula given by Bartlett and co-workers [1938] has shown a great constancy, although there was a considerable fall in the daily milk yield, thus indicating increase in vitamin A towards the end of the lactation.

The vitamin secreted in milk forms a very small percentage of that ingested even in the case of cows which are provided with enough of green roughages. This shows that, whereas feeds which are deficient in carotene will force the cow to deplete its reserves of vitamin A and ultimately lead to poorer milk, it is not possible to enrich it beyond a certain limit by feeding carotene-rich feeds. It is interesting to observe that each of the cows has yielded during the entire lactation about as much vitamin A as is equivalent to its one

TABLE I

The intake of green fodder and carotene and output of carotene and vitamin A in butter

Dated	Cow No. 1					Cow No. 2					Cow No. 3					Cow No. 4					Cow No. 5				
	Intake		Output			Intake		Output			Intake		Output			Intake		Output			Intake		Output		
	Green feed lb.	Carotene mg.	Butter gm.	Carotene mg.	Vit. A mg.	Green feed lb.	Carotene mg.	Butter gm.	Carotene mg.	Vit. A mg.	Green feed lb.	Carotene mg.	Butter gm.	Carotene mg.	Vit. A mg.	Green feed lb.	Carotene mg.	Butter gm.	Carotene mg.	Vit. A mg.	Green feed lb.	Carotene mg.	Butter gm.	Carotene mg.	Vit. A mg.
20-4-42	91	4080	526	2·63	3·37	91	4080	731	2·89	5·44	91	4080	702	4·84	6·66	81	3620	658	4·39	5·57	91	4080	511	..	..
30-4-42	90	4050	581	2·39	3·72	100	4500	740	3·32	4·74	110	4950	713	3·56	4·93	110	4950	570	2·55	4·09	72	3240	540	2·09	3·60
9-5-42	70	2804	684	2·76	4·73	70	2804	783	2·89	5·02	99	4005	687	3·16	4·05	89	3565	535	2·62	3·15	80	3200	640	2·72	3·77
14-5-42	77	2773	642	3·38	4·11	74	2631	644	4·54	3·80	99	3555	572	4·36	3·52	89	3200	587	2·99	3·31	84	2987	702	3·65	3·42
23-5-42	61	1520	593	3·12	4·41	63	1850	776	4·30	5·77	81	2370	615	3·74	5·20	77	2250	563	1·80	4·19	74	2185	584	3·56	5·39
29-5-42	6	932	615	2·85	4·42	64	994	663	3·83	5·61	75	1164	625	3·19	4·17	77	1195	539	2·15	3·18	75	1164	693	2·91	3·91
6-6-42	60	932	697	2·50	4·29	55	854	735	3·35	4·52	94	1459	623	3·06	4·15	88	1365	505	1·91	1·93	74	1148	644	2·57	2·48
13-6-42	46	714	435	1·74	2·79	66	1024	625	2·50	4·81	100	1553	545	3·27	3·91	91	1412	451	1·88	2·89	76	1179	553	2·42	3·69
20-6-42	65	1081	622	2·20	4·15	77	1281	682	3·29	4·55	127	2113	534	3·00	4·11	110	1830	438	0·95	3·59	113	1880	450	2·70	3·35
29-5-42	71	1181	616	3·17	4·42	74	1231	535	4·07	3·84	110	1830	582	3·03	4·33	88	1464	433	0·94	3·00	101	1680	570	2·91	4·09
4-7-42	78	1123	555	2·16	3·56	80	1151	582	3·26	3·73	117	1684	599	2·19	3·38	94	1353	452	0·75	2·90	110	584	466	2·70	3·59
11-7-42	65	936	463	2·23	2·75	60	792	528	3·23	3·65	99	1420	510	2·29	3·27	76	1094	412	0·87	2·02	94	1352	437	2·53	2 91
18-7-42	70	913	554	1·82	3·27	68	887	421	2·20	2·70	88	1147	433	1·69	3·00	78	1016	257	0·53	1·98	94	1238	436	1·87	2·91
27-7-42	67	873	515	2·53	3·83	Grass	..	450	2·15	3·81	88	1147	544	2·77	3·91	72	939	422	1·15	2·49	86	1121	409	1·83	1·57
1-8-42	67	904	640	1·87	3·77	..	..	429	..	3·63	88	1187	570	2·29	..	72	973	512	0·97	3·68	86	1160	516	1·68	4·50
8-8-42	50	674	490	2·00	3·39	64	863	396	2·36	2·95	67	904	436	1·82	3·01	84	1133	356	0·48	2·81	72	971	395	1·87	2·63
15-8-42	51	665	460	2·23	3·42	67	873	422	3·00	3·14	72	939	486	2·47	3·49	66	861	370	..	3·61	58	756	401	1·60	2·26
22-8-42	56	730	468	2·37	3·36	62	808	401	2·89	2·78	70	913	461	2·53	3·55	63	822	373	0·61	2·58	61	795	418	2·41	2·36
30-8-42	54	704	346	1·83	3·11	56	730	353	2·07	2·45	67	873	437	2·69	3·25	60	782	283	0·64	2·18	60	782	356	1·82	2·01
7-9-42	57	692	466	1·69	4·18	56	680	396	2·29	3·65	59	716	490	1·69	3·77	57	692	380	0·95	3·51	61	741	365	1·69	2 62
14-9-42	52	655	438	1·48	2·81	72	906	392	2·21	3·11	85	1070	483	1·32	3·10	70	881	370	..	3·42	48	604	441	1·55	3·28
22-9-42	81	1020	360	1·69	3·14	90	1133	304	1·93	2·89	95	1197	395	1·41	2·74	116	1460	300	0·68	2·39	75	944	355	0·68	2·18
28-9-42	80	1007	360	2·15	2·86	82	1032	272	1·66	2·86	93	1196	380	1·58	2·83	94	1184	331	0·85	2·46	77	969	362	1·05	2·60
3-10-42	80	1007	298	1·64	2·96	82	1032	329	2·12	2·78	83	1045	358	2·34	2·85	93	1171	285	0·79	2·56	70	881	298	0·83	2·45
13-10-42	82	1032	438	2·33	3·26	78	982	303	2·08	2·64	78	982	309	1·49	2·14	89	1120	381	0·99	2·93	65	818	347	0·83	2·22
19-10-42	83	821	404	1·78	2·49	84	831	334	2·27	3·00	82	811	328	1·67	2·69	83	821	381	0·95	3·02	67	663	309	0·82	2·45
25-10-42	94	972	..	1·91	..	80	828	334	2·30	..	89	921	341	2·14	..	92	952	374	..	..	72	745	322	..	..
2-11-42	92	952	334	1·61	2·74	92	952	321	2·08	2·96	107	1106	277	1·65	2·70	93	962	284	0·95	3·20	77	796	298	0·83	2·90
7-11-42	89	920	338	1·45	2·69	85	879	251	1·79	2·32	106	1096	262	1·34	2·89	91	941	327	1·16	4·02	90	931	277	0·90	2·91
14-11-42	84	869	353	2·60	2·80	85	879	247	2·42	2·53	104	1076	283	2·78	3·04	90	931	347	..	3·29	94	972	295	1·05	2·72
21-11-42	81	838	275	2·08	2·82	81	838	220	1·80	2·37	96	993	267	1·98	3·83	85	879	309	0·91	4·67	91	941	291	..	3·13
28-11-42	84	869	299	1·85	4·22	98	1013	254	2·23	3·71	110	1138	229	1·74	3·82	100	1034	114	0·42	2·05	105	1086	297	..	4·80

day's intake of carotene. If milk were to be regarded as the only source of vitamin A for man, then, in order to ensure the vitamin in an optimum quantity of about 4 mg. one will have to consume daily as much as 300 to 400 gm. of butter. Green leafy vegetables are a very rich source of vitamin A, and it is possible to have carotene separated in the laboratory from other plant sources like pasture grass and mixed with butter in such a quantity that say an ounce of it contains the daily dose. The economic and technical aspects of this problem and the extent of deterioration with time of such vitaminized butter or ghee require investigation and some work is already under way in this direction in these laboratories.

Table II gives the amount af vitamin A in Moore's Blue units per gram of fat for each of the five cows. It will be observed that this has been more or less constant over a considerable period, and it is towards the very end of the lactation that the value has gone to a considerably high figure, showing the increased richness of milk towards the end of lactation. The carotene content has not shown a similar increase (per gram of fat) towards the end of lactation. A reference to Table II will show that only in cows Nos. 2 and 3 is there any definite indication of an increase. The butter prepared in bulk from the milk of cows Nos. 4 and 5 appeared even by visual observation to be very much lighter in colour than the other samples.

TABLE II

Vitamin A in blue units and carotene in microgrammes per gram of fat

Date	Cow No. 1		Cow No. 2		Cow No. 3		Cow No. 4		Cow No. 5	
	Vit. A in B. P.	Carotene in mg.	Vit. A in B. U.	Carotene in mg.	Vit. A in B. U.	Carotene in mg.	Vit. A in B. U.	Carotene in mg.	Vit. A in B. U.	Carotene in mg.
20-4-42	25	4·73	29	4·01	37	6·90	33	6·67	..	..
30-4-42	25	4·11	25	4·40	27	4·99	28	4·47	26	3·87
9-5-42	27	4·03	25	3·69	23	4·60	23	4·90	23	4·25
14-5-42	25	5·27	23	5·54	24	7·62	22	5·09	19	5·20
23-5 42	29	5·26	29	5·54	33	6·80	29	3·20	36	6·10
29-5-42	28	4·63	33	5·78	26	5·10	23	3·99	22	4·20
6-6-42	24	3·59	24	4·56	26	4·91	15	3·78	15	3·99
13-6-42	25	4·02	30	4·00	28	5·90	25	4·17	26	4·38
20-6-42	26	3·54	26	4·82	30	5·62	32	2·17	29	6·00
29-6-42	28	5·15	28	7·61	29	5·21	27	2·17	28	5·10
4-7-42	25	3·89	25	9·76	22	3·71	25	2·11	30	6·00
11-7-42	24	4·82	27	6·12	25	4·49	19	1·66	26	5,79
18-7-42	23	3·29	25	5·23	27	3·90	30	2·06	26	4·29
27-7-42	29	4·91	33	4·78	28	5·00	23	2·72	15	4·47
1-8-42	23	2·92	33	..	35	4·02	28	1·90	34	3·26
8-8-42	27	4·08	29	5·96	27	4·13	31	1·35	26	4·73
15-8-42	29	5·00	29	7·14	28	5·63	38	..	22	3·99
22-8-42	28	5·06	27	7·21	30	5 49	27	2·15	22	5·77
30-8-42	35	5·29	27	8·18	29	6·16	30	2·26	22	5·11
7-9-42	35	2·44	36	5·78	30	3·45	36	2·50	28	4.63
14-9-42	25	3·38	31	5·64	25	2·73	36	..	29	3·51
22-9.42	34	4·69	37	6·35	27	3·57	31	2·27	24	1.92
28-9-42	31	5·97	41	6·10	29	4·16	29	2·57	28	2·90
3-10-42	29	5·50	33	6·44	31	6·54	35	2·77	32	2·79
13-10-42	29	5·32	34	6·87	27	4·92	30	2·60	25	2·39
19·10-42	24	4·41	35	6·80	32	5·09	31	2·49	31	2·65
2-11-42	32	4·82	36	6·48	38	5·96	44	3·34	38	..
7-11-42	31	4.29	36	7·13	43	5·11	48	3·55	41	3·25
14-11-42	31	4·37	40	9·80	31	9·82	37	..	36	3·56
21-11-42	40	7·56	42	8·18	56	7·42	59	2·94	42	..
28 11·42	55	6·19	57	8·78	65	7·60	70	3·68	63	..

The amounts of vitamin A in Blue units per 100 c.c. of milk are shown in Table III. The concentration of vitamin A has been fairly constant throughout the major part of the lactation, but towards the end there is a sharp rise in the blue values. This is the period when milk develops abnormalities in composition, like increased salt content. A glance at the carotene figures shows that in cows Nos. 1-3, the figures have gone up towards the end, but in cows 4-5, there is no such tendency. This shows the individual variability of the cow as a converter of feed carotene to butter carotene.

TABLE III

Vitamin A (in Moores blue units and carotene in micrograms, Y) per 100 c.c. of milk

Date	Cow No. 1		Cow No. 2		Cow No. 3		Cow No. 4		Cow No. 5	
	Vit. A	Carotene	Vit. A	Carotene	Vit. A	Carotene	Vit. A	Carotene	Vit. A	Carotene
20-4-42	125	25	138	19	167	31	150	30	..	..
30-4-42	126	21	124	22	125	24	132	29	128	19
9-5-42	155	23	143	21	113	23	109	23	113	20
14 5-42	143	30	100	31	100	32	113	26	100	27
23-5-42	163	30	160	31	150	28	147	16	150	25
29-5-42	150	25	170	30	125	25	113	20	107	21
6-6-42	160	24	130	25	135	26	80	20	75	20
13-6-42	125	20	150	20	140	30	120	20	125	21
20-6-42	150	24	150	28	154	26	145	9	137	24
29-6-42	157	32	130	35	137	25	125	10	113	24
4-7-42	137	21	125	28	113	19	120	8	113	22
11-7-42	125	25	130	30	115	21	100	11	100	22
18-7-42	150	28	115	24	125	18	100	7	90	17
27-7-42	180	31	150	22	150	27	125	15	58	17
1-8-42	130	21	150	36	175	22	150	16	165	16
8-8-42	175	27	137	28	150	23	140	6	100	18
15-8-42	182	30	140	34	150	27	187	19	100	18
22-8-42	175	32	125	28	150	38	130	8	100	26
30-8-42	175	26	125	27	150	32	130	10	100	23
7-9-42	200	21	162	26	162	18	175	12	113	19
14-9-42	150	20	150	27	139	15	175	18	158	29
22-9-42	175	24	150	26	150	20	138	10	113	9
28-9-42	163	31	150	22	138	20	135	12	125	13
3-10-42	180	26	150	29	131	32	163	13	138	12
13-10-42	175	32	163	33	163	29	163	14	150	12
19 10-42	163	30	175	34	163	26	175	14	150	13
25-10-42	155	28	160	30	150	32	167	16	163	16
2-11 42	175	26	195	35	200	31	200	15	163	12
7-11-42	200	24	188	37	200	24	232	17	175	14
14-11-42	175	42	200	49	163	51	188	26	163	16
21-11-42	225	43	225	44	300	40	300	15	200	..
28-11-42	300	34	325	50	325	36	400	21	300	..

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SUMMARY

(1) A study of the vitamin A and carotene content of milk from five Sahiwal cows was made throughout their lactation.

(2) The cows were on liberal green ration getting up to 4,000 mg. of carotene; but the daily output of vitamin A and carotene in milk was about 4-5 mg. only.

(3) Towards the end of the lactation there was a rise in the vitamin A and carotene content of the milk, although the total output, due to fall in the milk yield, was not much affected.

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RECTAL TEMPERATURES OF CERTAIN ANIMALS AT REST

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(With four text-figures)

IN view of the variable and frequently high air temperatures in India and the effect of air temperature on body temperature of resting animals, it is important to have as precise knowledge as possible on the normal body temperature under different conditions. This is particularly so with relatively homothermous animals, i.e. ones whose heat regulation is less perfect than in man.

LITERATURE

The normal range of rectal temperature for various domesticated animals in health in temperate countries has been laid down by numerous observers. For animals in hotter countries there must be fewer records. To obtain an accurate idea of an animal's temperature important points are that the thermometer must be properly used and the observations repeated throughout the 24 hours. Lazarus-Barlow [1928] has shown with rabbits how incorrect an impression may be formed of the normal swing of the body temperature, unless observations are made at intervals as short as, say four hours. Varrier-Jones and Sims Woodhead [1915] devised a special apparatus by which the rectal temperatures of cattle could be recorded mechanically and in a quasi-continuous manner. In this way they were able to show well-marked variations during the 24 hours. During most of the day the temperature lies between 101 and 102 °F. It is always highest during the day when the animal is standing or moving about or has just been fed, and falls slowly during the night to reach its minimum [100.7°F.] about 3-5 a.m. Milking almost always caused a slight rise. The highest points were at 8-9 a.m. and 4-6 p. m., the diurnal range being about 1.2°F. Wooldridge [1905] had likewise found that for cattle in Ireland the average morning and evening temperatures are 101.5 and 102°F Hobday [1896] gives figures of 101.3 and 101.8°F. for cattle in England.

With animals in hot countries the matter is somewhat different. As pointed out by Hornby [1942], the expression 'normal temperature', as applied to animals under such conditions, has only a provisional meaning. Regan and Freeborn [1936] found that high-producing Jersey cows kept at air temperature of 85°F. or over for more than 24 hours were unable to control their body temperatures. Regan and Richardson [1938] found that the rectal temperatures of cattle remained constant at 101-101.3°F. when the air temperature was between 40 and 70°F., above that it began to increase and at 100°F. the rectal temperature was 105·1°F. Although cattle native to hot countries are more tolerant of high air temperatures, principles similar to those just enunciated hold good. Their normal resting temperatures do not however differ from cattle of cooler countries. For instance, Manresa and Gomez [1937] for cattle of the Nellore breed in the Philippines give a mean daily value of 38.66°C. (101.59° F.), the diurnal and nocturnal values being 38.7°C. (101.66°F.) and 38.56°C (101.4°F.). These figures were based on four-hourly readings taken over seven days in April. The values were lowest at 6 a.m. and highest at 6 p.m., while the diurnal values were said to be significantly higher than the nocturnal (number of observations not stated). They too note a high and significant correlation at the same hours of the day between body temperature and air temperature. In a later paper by Manresa and Falcon [1939] for six mature Nellore cattle higher values are given, viz. average 39.01°C. (102.2°F.) during the hotter part of the year, March to July (average air temperature 28.22°C. or 82.8°F.) and average 38.82°C. (101.88°F.) during the cooler months—August to February (average air temperature 26.87°C. or 80.37°F.).

As for buffaloes, there is the same correlation with air temperature. MacGregor [1941] says the normal temperature may be put at 98.8°F. but that on hot days it may reach 104°F. For sheep, Ritzman and Benedict [1931] give rectal temperatures of some 50 animals at different times. The ranges found were:

for light-woolled yearlings 39·2-40·40°C. (102·56-104.72°F.)

for light-woolled adults 38·8-39·7°C. (101·84- 103.46°F.)

for heavy-woolled adults 38·9-39·7°C. (102-103·46°F.)

They state that sheep are not sensitive to environmental temperatures, presumably owing to protection from the fleece. Probably, however, they were not referring to higher air temperatures. For goats in India (Lahore), Sadiq [1943] has recently given some figures. The average morning temperature for 366 goats was 100.7°F., 70 per cent of the observations falling between 100 and 102°F., the average evening temperature of 230 goats was 102.2°F., 75 per cent of the observations being in the range 101-103°F. Air temperature has a marked effect, the curves for body temperature and mean monthly air temperatures roughly coinciding.

For poultry, Fronda [1925], using White Leghorn fowls kept in air at 60-80°F., noted a sharp fall of body temperature between 6 and 8 p.m. to the lowest point from 10 p.m. to midnight. The temperature then rose and attained its highest level from 8 a.m. to 6 p.m. Examples are: 6 p.m. 107.28°F., 10 p.m. 105.75°F., 8 a.m- 107.4°F. The mean 6 a. m. to 6.p.m. level was from 106.83 to 107.36°F. The variation in level is attributed to feeding activities in the morning and afternoon and the habit of roosting at about 6 p.m. Air temperatures were not considered to affect the level, but no observations were made at temperatures above 80°F. Heywang [1938] has also dealt with the relationship between air temperature and body temperature in hens. In the case of White Leghorn hens kept in cages and at air temperature which varied from an average of 54° to 99° F. at different observations the mean morning body temperatures (8-10 a.m.) fell from 106.3 to 105.6°F., in passing from lower to higher air temperature, while the mean afternoon temper-

atures (3-5 p.m.) increased from 106.4 to 108.6°F., so that the difference between the a.m. and p.m. averages at the highest air temperature was as much as 3.0° F. From two-hourly body temperature recordings, taken over periods when the average room temperature ranged from about 57 to 75°F., it was shown that body temperatures were highest at 4 a.m, and lowest at midnight (cf. Fronda above), the difference being about 1·5°F. The general finding therefore was that body temperatures increased and decreased in nearly direct relation to the air temperature. The greatest change in body temperature occurred between 6 and 8 p.m., with a decrease of about 0·8°F.

Experimental conditions

The experiments were made on male buffaloes, cows of theHariana breed, sheep, goats and fowls at Izatnagar and on Kumauni hill-bulls and sheep at Mukteswar. The animals selected appeared to be in reasonably good health, but a few troubles developed during the experiments. They were housed in sheds sheltered by trees and were not crowded. In winter some straw bedding was provided. They were watered and fed at times that would not interfere with the observations. The cattle were given an adequate ration; cows received a concentrated mixture usually containing ground-nut cake, rape-cake, wheat bran, gram husk, with mineral supplement, while the roughage consisted of hay or *bhusa*, fresh green fodder or silage according to the season. Buffaloes got gram or cowpeas and maize or *jowar* with the concentrates. Hill-bulls got a ration similar to the cows, while the sheep and goats also got a suitable concentrate and roughage ration.

With the cattle and buffaloes five series of observations lasting 10, 10, 13, 12 and 10 days were made in April, June, July, August and December 1941, the temperatures being recorded at 7 and 10 a.m., 1, 4, 7 and 10 p.m. and occasionally at 1 and 4 a.m. In April, July and December in case of buffaloes supplementary figures for 7 a.m. and 4 p.m. were taken from another experiment. With sheep and goats at Izatnagar there were two series each of five days in February and June 1943, and with fowls, two series each of three days in February and (end) May 1943. At Mukteswar hourly temperature observations extending over 24 hours were made on hill-bulls and sheep during July 1942. Dry-bulb temperatures were recorded with a hygrometer suspended at the centre of the shed or room at about eight feet from the ground.

The following details are necessary.

Buffaloes. Five male Indian river buffaloes of 'country' breed, 6-8 years old, and weighing 950 to 1,100 lb. were used. They were housed in a *chapper* open at the sides. They were watered daily at 7-30 a.m. and 3 p.m. and fed at 10-30 a.m. and 4-30 p.m. Slight exercise was given at 7-30 a.m. Two of them suffered from slight diarrhoea for a few days during the first experiment (April) and one of them had to be replaced before the next experiments.

Cows. Five in number, ages in years: 3, 4, 10, 11 and 14, body weights 870-1,100 lb. Two of them calved in February 1941 and were in milk during the first three experiments; before the fifth experiment (December) one of them went dry and two others had calved in September and October. They were tied in a shed, which was closed on three sides, except from 7 to 10 a.m. when they were tied in the open. The only exercise was a short walk after 10 a.m. Watering was at 7-30 a.m. and 3 p.m.; feeding at 5 a.m. and 3. p.m. at which times they were milked. The air temperature in the shed was about the same as that in the buffalo-shed. All five remained in normal health, except that the youngest had a slightly raised temperature for 1½ days during the second experiment. The third and fourth experiments (July, August) coincided with the monsoon and rain totalling 11·07 inches fell on nine of the 25 days in these experiments. The temperature of the rain water was 75-78°F. During the fifth experiment the other

cows of the milking herd were also housed in the shed from 2 p.m. to 7-30 a.m.

Sheep (at Izatnagar). Ten male sheep of 'country' breed were used, all 1-1½ years old. At the first experiment their body weights were 36-44 lb. and at the second 44-60 lb. They were tied about 2 feet apart in a shed open on the east side. They had not been recently shorn.

Goats. Ten were used in each series of experiments; before the second experiment (June) there were three replacements. Four were males and nine were females, and all but one were 1½ years old, one being four years old. At the first experiment their weights were 26-40 lb. and at the second 30-52 lb. They were housed with the sheep, and as with sheep water was offered after every temperature-recording.

Fowls. Nine cocks, 1 year old and about 5 lb. in weight, were used, mostly White Leghorn-*desi* cross. They were kept in separate cages in a large laboratory, the doors and windows of which were kept open from 10 a.m. to 4 p.m. during the May experiment. Food and water were always available in the cage.

Sheep (at Mukteswar). Nine sheep, 1-1½ years of age, were selected as being in reasonable health, though three of them had 'snuffles'. They had been shorn eleven days before the experiment. They were kept untethered in a shed, the air temperature of which was maintained at 69-74°F.

Hill-bulls. Ten were used and they were housed in the shed which had been used for the sheep. The air temperature was maintained at 70-75°F. They were watered three hours before the experiment was started and immediately afterwards. Hay was fed at 6 a.m.

PRELIMINARY EXPERIMENTS

The personal factor in temperature-taking

This is a matter, the importance of which cannot be too often stressed, especially since the work is frequently placed in the hands of uninstructed subordinates. The technique of individuals varies, both in respect of the time the thermometer is allowed to remain in the rectum and the depth to which it is inserted. In order to check up these points some experiments were arranged. In one of these, six observers, all professionally qualified, were engaged to take the temperatures of five cows and five buffaloes, one observer (F.C.M.) acting as control. None of the others was informed of the object of the experiment, but an instruction was given that the thermometer was to remain in the rectum for exactly one minute from the time it was considered to be in position. The observers passed from one animal to another as quickly and as quietly as possible and no observer was aware of the readings of the others. All the thermometers were checked before use in water at 97°, 101° and 104°F. and showed differences of not more than 0·2°F. and usually not more than 0·1°F.

With the cows, the differences between the highest and lowest readings were: 1·3, 0·9, 0·2, 1.6, and 0·5°F. (mean 0·9°F.) and with the buffaloes 2·0, 1·2, 0·9, 1·1 and 1·4°F. (mean 1·3°F.). The differences between the highest and next lowest readings were 0·8, 0·7, 0·1, 0·9, 0·3°F. (mean 0.56° F.) with cows and 1·4, 0·8, 0·5, 0·7, 0·8°F. (mean 0·84° F.) with buffaloes. One observer gave almost consistently the lowest readings, obviously because he had not inserted the thermometer to its full depth.

The next experiment was designed to show the effect of inserting the thermometer to different depths. Ten cows were used and most of the work was done by one observer. Differences from the true temperature (i.e. when thermometer was fully inserted) were

1. when little more than the bulb was inserted, range 0·3 to 1·5°F. (mean 0·6F.),

2. when the bulb was inserted an extra cm.—range 0·1 to 0·6°F. (mean 0·3°F).

These facts, which merely confirm some observations made long ago by Hobday [1896], require no comment. Incidentally, when the air temperature is higher than the rectal temperature, the bulb of the thermometer should be covered with damp cotton-wool the moment the instrument

TABLE I

Body temperatures (°F) of buffaloes, cows, sheep, goats and fowls

Hour	Mean	Min.	Max.	A. T.	Obs.	Mean	Min.	Max.	A. T.	Obs.
					Buffaloes					
	April					June				
7 A. M. ..	99·74	98·0	101·3	No record	70	100·25	99·0	102·0	81-87	50
10 „ ..	100·18	98·0	103·2		50	100·25	98·0	101·9	87-97	50
1 P. M. ..	100·84	98·8	102·7		50	100·53	99·0	102·0	90-104	50
4 „ ..	101·50	100·0	104·0		62*	101·32	99·0	103·2	94-108	49*
7 „ ..	101·79	99·0	103·4		43*	102·02	100·2	103·2	86-102	49*
10 „ ..	101·22	98·2	103·5		48*	101·19	98·7	102·8	81-95	49*
1 A. M. ..	100·95	99·4	102·0		20	101·35	99·8	102·4	85-93	19*
4 „ ..	100.65	99·2	101·6		20	101·20	99·6	102·6	82-88	19*
	July					August				
7 A. M. ..	100·15	98·0	102·4	80-86	105	100·12	98·4	101·8	77-85	60
10 „ ..	100·25	99·0	102·6	84-98	65	100·37	98·2	101·6	78-93	60
1 P. M. ..	100·93	98·6	102·8	89-104	65	100·78	99·2	102·0	80-102	60
4 „ ..	101·51	98·4	103·0	82-106	105	101·02	99·6	102·6	80-102	60
7 „ ..	101·32	99·4	103·4	85-101	65	100·69	99·4	102·5	80-92	60
10 „ ..	101·31	99·6	103·5	81-94	65	100·75	99·4	102·0	79-89	60
1 A. M. ..	101·92	101·0	103·2	82-88	20	100·82	99·6	101·8	76-84	20
4 „ ..	101·42	100·0	102·8	80-83	20	100·43	99·4	101·4	73-77	20
							Cows			
	December					April				
7 A. M. ..	98·53	96·0	101·0	49-60·5	85	100·97	99·4	102·2	No record	50
10 „ ..	98·47	95·4	100·8	58-69	50	101·07	99·8	101·9		50
1 P. M. ..	99·20	96·2	101·4	70-80	50	101·31	100·1	103·3		50
4 „ ..	100·43	98·4	102·6	69-80	85	101·62	100·3	102·8		50
7 „ ..	101·23	99·4	102·4	65-78	50	101·97	100·4	103·2		50
10 „ ..	100·96	98·6	103·0	59-66·5	50	101·70	100·7	102·8		50
1 A. M. ..	..	..	..	..	..	101·50	100·6	102·0		20
4 „ ..	..	..	..	..	..	101·32	100·6	102·0		20

* Abnormal temperatures due to sickness or unknown causes have been omitted.

Table I—*contd.*

Hour		Mean	Min.	Max.	A. T.	Obs.	Mean	Min.	Max.	A. T.	Obs.
					Cows						
				June					July		
7 A. M.	..	101·40	99·4	104·3	81-87	50	101·14	100·0	102·2	81-88	65
10 „	..	101·18	99·8	103·6	87-97	49*	101·17	100·0	102·0	85-97	65
1 P. M.	..	101·15	99·4	102·4	88-102	49*	101·37	100·3	102·4	88-103	65
4 „	..	101·50	100·7	103·4	92-106	50	101·56	100·6	102·6	91-105	65
7 „	..	101·61	100·5	103·3	88-102	49*	101·57	100·4	103·0	85-101	65
10 „	..	101·62	100·4	103·6	81-97	48*	101·45	100·2	103·0	83-95	65
1 A. M.	..	101·65	100·2	103·0	86-94	19*	101·25	100·0	102·7	84-90	20
4 „	..	101·50	100·0	102·8	83-89	19*	100·91	100·0	102·4	82-86	20
				August					December		
7 A. M.	..	100·69	99·6	101·8	77-85	60	100·80	98·8	102·0	48-59	50
10 „	..	100·96	99·5	101·7	78-93	60	100·19	97·4	101·6	56-66	50
1 P. M.	..	101·17	99·8	102·4	79-101	60	100·66	98·6	101·8	66-78	50
4 „	..	101·19	100·0	103·4	80-102	60	101·35	99·6	102·6	70-78	50
7 „	..	101·10	100·0	102·5	80-92	60	101·90	100·0	103·6	65-77	50
10 „	..	101·01	100·0	102·4	79-90	60	101·66	100·0	103·0	58-66·5	50
1 A. M.	..	100·84	100·2	102·0	76-81	20	..	..	..	..	..
4 „	..	100·55	100·0	101·6	73-78	20	..	..	..	..	..
					Sheep						
				February					June		
7 A. M.	..	101·54	99·0	102·9	48-51·5	50	103·87	103·0	104·7	80-94	50
10 „	..	102·06	99·0	103·2	57-60	50	103·47	102·0	104·6	92-99	50
1 P. M.	..	102·57	100·5	103·6	69-70	50	103·85	102·6	105·	98-106	50
4 „	..	102·64	101·4	103·8	69-74	50	103·60	102·2	104·8	105-109	50
7 „	..	103·11	102·0	104·2	62-73·5	50	104·06	103·2	105·0	101-105	50
10 „	..	102·52	101·2	104·0	59-67	50	103·77	102·8	106·0	90-99	50

* Abnormal temperatures due to sickness or unknown causes have been omitted.

TABLE I—*concld.*

Hour	Mean	Min.	Max.	A. T.	Obs.	Mean	Min.	Max.	A. T.	Obs.
					Goats					
			February					June		
7 A. M. ..	101·83	100·4	104·4	48-51·5	49*	104·15	101·0	106·4	80-94	50
10 „ ..	101·52	99·8	103·6	57-60	49*	104·28	101·4	106·6	92-99	50
1 P. M. ..	102·50	99·2	105·8	69-70	49	104·97	102·8	107·0	98-106	50
4 „ ..	103·48	101·5	106·4	69-74	49*	104·87	103·1	106·8	105-109	50
7 „ ..	103·65	101·8	105·8	62-73·5	49*	105·27	104·0	107·0	1011·05	50
10 „ ..	103·20	100·4	105·6	59-67	49*	105·22	103·6	107·6	90-99	50
					Fowls					
			February					May		
7 A. M. ..	106·85	104·8	108·3	63-66	27	108·25	107·0	109·2	92-94	27
10 „ ..	106·79	105·7	108·0	70-73·5	27	107·97	106·6	109·0	93-95	72
1 P. M. ..	106·80	105·8	108·3	74-74·5	27	107·83	106·6	109·0	95-98	27
4 „ ..	106·94	105·8	108·3	75-76·5	27	107·78	106·6	109·0	98-99	27
7 „ ..	106·13	104·8	107·7	74·5-75·5	27	106·96	106·0	108·3	94-95	27
10 „ ..	105·23	104·2	106·7	73·5-75	27	106·40	106·0	107·6	92-93	27

* Abnormal temperatures due to sickness or unknown causes have been omitted.

is withdrawn and until the reading is taken.

It would have been preferable for one observer to make the readings recorded in this paper but this was not feasible. However, most of them were done by one person and care was always taken that the worker carried out observations properly.

Effect of defaecation on temperature recordings

In experiments at Izatnagar in March and May 1943, 51 observations were made on 27 cows by the one observer. Temperatures were recorded every few minutes, and as soon as an animal had defaecated, its temperature was taken again. The difference, if any, between this recording and the next previous pre-defaecation recording was noted. In 43 instances the post-defeacation temperature was lower by 0·3°F., mean (range 0·1-0·8°F), in six both readings were identical and in only

TABLE IA

Body temperatures (°F.) of hill-bulls and sheep (at Mukteswar)

Hour		Sheep Month: July			Hill-bulls Month July		
		Mean	Min.	Max.	Mean	Min.	Max.
2 P. M.	..	103·09	102·8	103·6	101·73	100·6	102·4
3 „	..	103·13	103·0	103·6	102·10	101·2	102·4
4 „	..	103·18	102·6	103·6	102·07	101·4	102·4
5 „	..	103·31	102·8	103·6	102·04	101·2	102·6
6 „	..	103·66	103·2	104·2	101·79	101·2	102·5
7 „	..	103·47	103·0	104·2	101·65	101·0	102·0
8 „	..	103·07	102·0	103·8	101·43	100·8	102·0
9 „	..	102·98	101·8	103·8	101·26	100·4	101·8
10 „	..	102·96	101·8	103·4	101·12	100·6	101·8
11 „	..	102·56	101·4	103·0	100·96	100·2	101·6
12 night	..	102·62	101·2	103·6	100·94	100·0	102·0
1 A. M.	..	102·51	101·6	103·0	100·68	100·0	101·2
2 „	..	102·77	101·7	103·1	100·91	100·0	101·4
3 „	..	102·79	102·1	103·4	100·87	99·7	101·6
4 „	..	102·78	102·2	103·4	100·62	99·4	101·2
5 „	..	102·69	102·1	103·2	100·92	100·4	101·2
6 „	..	102·49	102·0	103·0	100·99	100·5	101·3
7 „	..	102·74	102·2	103·5	101·68	101·2	102·0
8 „	..	103·04	102·4	103·6	101·82	101·4	102·2
9 „	..	103·11	102·5	103·6	102·14	101·8	102·5
10 „	..	102·93	102·1	103·5	101·99	101·7	102·4
11 „	..	103·02	102·3	103·6	101·92	101·5	102·4
12 noon	..	102·93	102·5	103·4	101·99	101·4	102·6
1 P. M.	..	103·01	102·4	103·6	101·74	101·1	102·3

Air temperature 69-74°F.
Nine observations at each hour

Air temperature 70-75°F.
Ten observations at each hour

TABLE IB

Body temperatures (°F.) of buffaloes, cows and bullocks

Animal	Month	Hour	Mean	Min.	Max.	A. T.	Obs.
Buffaloes	April ..	Noon ..	100·07	98·0	102·0	No record	15
	May ..	7 A. M. ..	99·10	97·8	101·5	75-89	70
	„ ..	11 „ ..	99·63	97·0	103·1	91-105	75
	„ ..	Noon ..	100 08	98·9	101·6	102-107	25
	„ ..	4 P. M. ..	101·73	100·2	104·0	99-112	70
	June ..	Noon ..	99·41	99·0	99·8	90-98	15
	July ..	11 A. M. ..	100·13	99·0	101·4	88-88·5	10
	August ..	11 „ ..	99·60	97·6	100·8	82-91	65
	December ..	11 „ ..	97·26	96·0	99·8	54-67	75
Cows	April ..	Noon ..	100·69	100·1	101·4	No record	15
Bullocks	April ..	7 A. M. ..	101·30	100·6	101·8	75-82	20
	„ ..	4 P. M. ..	101·86	101·2	103·5	102-109	20
	May ..	7 A. M. ..	101·38	99·6	103·0	77-89	70
	„ ..	11 „ ..	101·49	100·0	102·7	91-105	75
	„ ..	Noon ..	102·09	101·0	102·7	102-107	25
	„ ..	4 P. M. ..	102·03	101·1	103·0	100-112	70
	June ..	Noon ..	100·87	100·2	101·4	90-98	15
	July ..	7 A. M. ..	101·91	100·5	103·0	80-84	40
	„ ..	11 „ ..	102·24	100·7	104·0	88-88·5	10
	„ ..	4 P. M. ..	102·65	101·7	103·7	82-89	40
	August ..	11 A. M. ..	102·29	101·0	104·8	82-91	65
	December ..	7 A. M. ..	99·87	97·8	100·8	49-53·5	35
	„ ..	11 „ ..	100·21	99·0	101·8	54-67	75
	„ ..	4 P. M. ..	101·66	100·8	102·5	69-76	35

TABLE IC

Body temperature of buffaloes

Month: August.
Time: 8—9 A. M.
Air temperature: 79·5—86·5°F.
744 observations on 38 animals over 20 days.

2	(0·27 per cent)	observations were from	96·0 to 96·5°F.
7	(0·94 „ „)	„ „ „	96·6 „ 97·1°F.
15	(2·02 „ „)	„ „ „	97·2 „ 97·7°F.
31	(4·17 „ „)	„ „ „	97·8 „ 98·3°F.
58	(7·80 „ „)	„ „ „	98·4 „ 98·9°F.
116	(15·59 „ „)	„ „ „	99·0 „ 99·°F.
206	(27·68 „ „)	„ „ „	99·6 „ 100·1°F.
151	(20·30 „ „)	„ „ „	100·2 „ 100·7°F.
112	(15·05 „ „)	„ „ „	100·8 „ 101·3°F.
43	(5·78 „ „)	„ „ „	101·4 „ 101·9°F.
3	(0·40 „ „)	„ „ „	102·0 „ 102·5°F.

Thus, 585 (78·62 per cent) were from 99·0 and 101·3°F.

Mean of observations=99·97°F.
Minimum .. =96·0°F.
Maximum .. =102·0°F.

TABLE II

Ranges of body temperature (°F.) of various animal species

Species	Month	Hour	Whole range and per cent obs.	Subranges and per cent obs.	Air temperature
	April	7-10 A. M.	98·6—100 9 (82)	98·6—100·3 (65); 100·4—100·9 (17)	No record
	"	1-4 P. M.	100·0—102·9 (84) @	100·0—101·7 (60); 101·8—102·9 (24)	do.
	"	7-10 "	100·6—103·5 (79)	100·6—101·7 (30); 101·8—102·3 (23); 102·4—102·9 (26)	do.
	"	1-4 A. M.	100·6—101·1 (53)	..	do.
	June	7-10 "	99·0—101·3 (90)	99·0— 99·5 (19); 99·6—101·3 (71)	81—97
	"	1-4 P. M.	99·0—101·9 (89) @	99·0—100·1 (27); 100·2—101·3 (46); 101·4—101·9 (16)	90—108
	"	7-10 "	100·8—102·5 (77)	100·8—101·3 (16); 101·4—101·9 (28); 102·0—102·5 (33)	81—102
	"	1-4 A. M.	101·0—101·5 (42)	..	82—93
	July	7-10 "	99 0—101·9 (96) *	99·0— 99·5 (12); 96·6—100·7 (69); 100·8—101·9 (15)	80—98
	"	1-4 P. M.	100·2—102·5 (87)	100·2—100·7 (13); 100·8—101·9 (61); 102·0—102·5 (13)	89—106
Buffaloes	"	7-10 "	100·0—102·3 (86)	100·1—101·1 (38); 101·2—101·7 (30); 101·8—102·3 (18)	81—101
	"	1-4 A. M.	101·2—101·3 (60)	..	80—88
	August	7-10 "	99·0—101·3 (91) *	99·0— 99·5 (16); 99·6—100·7 (64); 100·8—101·3 (11)	77—93
	"	1-4 P. M.	99·8—102·1 (93)	99·8—100·3 (18); 100·4—101·5 (60); 101·6—102·1 (15)	80—102
	"	7-10 "	100·0—101·7 (83)	100·0—100·5 (38); 100·6—101·7 (45)	79—92
	"	1-4 A. M.	100·2—101·3 (70)	..	73—84
	December	7-10 "	96·2—99·7 (82) *	96·2— 96·7 (12); 96·8— 97·9 (37); 98·0— 99·7 (33)	49—69
	"	1-4 P. M.	98·6—102·1 (85)	98·6— 99·1 (9); 99·2—100·9 (55); 101·0—102·1 (21)	69—80
	"	7-10 "	100·0—102·3 (83)	..	59—78
	"	1-4 A. M.	..	..	..
	April	7-10 A. M.	100·6—101·7 [69]	..	No record
	"	1-4 P. M.	100·1—102·4 [97] @	100·1—100·6 [15]; 100·7—101·8 [65]; 101·9—102·4 [17]	do.
	"	7-10 "	101·0—102·7 [91]	101·0—101·5 [20]; 101·6—102·1 [49]; 102·2—102·7 [22]	do.
	"	1-4 A. M.	100·6—102·3 [100]	..	do.
	June	7-10 "	100·0—102·3 [94]	100·0—101·1 [29]; 101·2—101·7 [47]; 101·8—102·3 [18]	81—97
	"	1-4 P. M.	100·6—102·3 [87]	100·6—101·1 [18]; 101·2—101·7 [56]; 101·8—102·3 [13]	88—106
	"	7-10 "	100·5—102·8 [95]	100·5—101·0 [15]; 101·1—102·2 [69]; 102·3—102·8 [11]	81—102
	"	1-4 A. M.	100·8—102·5 [74]	..	83—94
	July	7-10 "	100·8—101·9 [84]	..	81—97
Cows	"	1-4 P. M.	100·9—102·0 [88]	..	88—105
	"	7-10 "	101·0—102·1 [90]	..	83—101
	"	1-4 A. M.	100·6—101·7 [63]	..	82—90
	August	7-10 "	100·2—101·9 [88]	100·2—101·3 [66]; 101.4—101·9 [22];	77—93
	"	1-4 P. M.	100·4—102·1 [87]	100·4—100·9 [23]; 101·0—101·5 [44]; 101·6—102·1 [20]	79—102
	"	7-10 "	100·1—101·8 [91]	..	79—92
	"	1-4 A. M.	100·2—101·3 [80]	..	73—81

TABLE II—*contd.*

Species	Month	Hour	Whole range and per cent obs	Subranges and per cent obs	Air temperature
Sheep	December	7-10 ,,	100·0—101·7 [74]	100·0—100·5 [19]; 100·6—101·1 [32]; 101·2—101·7 [23]	48—66
	,,	1-4 P. M.	100·4—102·1 [81]		66—78
	,,	7-10 ,,	100·6—102·9 [91]	100·6—101·1 [11]; 101·2—102·3 [65]; 102·4—102·9 [15]	58—77
	February	7-10 A. M.	100·8—103·1 [84]	100·8—101·3 [12]; 101·4—102·5 [55]; 102·6—103·1 [17]	48—60
	,,	1-4 P. M.	101·7—103·4 [87]	101·7—102·2 [17]; 102·3—102·8 [42]; 102·9—103·4 [28]	69—74
	,,	7-10 ,,	102·0—104·3 [93]	102·0—102·5 [27]; 102·6—103·1 [35]; 103·2—104·3 [31]	59—73·9
	June	7-10 A. M.	103·0—104·7 [94]	103·0—103·5 [34]; 103·6—104·1 [41]; 104·2—104·7 [19]	80—99
	,,	1-4 P. M.	103·1—104·8 [86]	103·1—103·6 [41]; 103·7—104·8 [45]	98—109
	,,	7-10 ,,	103·2—104·9 [86]	103·2—104·3 [69]; 104·4—104·9 [17]	90—105
Goats	February	7-10 A. M.	100·4—103·3 [88]	101·4—102·1 [65]; 102·2—103·3 [23]	48—60
	,,	1-4 P. M.	101·6—104·5 [80]	101·6—102·7 [31]; 102·8—103·3 [25]; 103·4—104·5 [24]	69—74
	,,	7-10 ,,	101·8—105·3 [86]	101·8—102·9 [23]; 103·0—103·5 [35]; 103·6—105·3 [28]	59—73·5
	June	7-10 A. M.	103·4—105·7 [76]	103·4—103·9 [19]; 104·0—104·5 [32]; 104·6—105·7 [25]	80—99
	,,	1-4 P. M.	104·0—106·3 [75]	104·0—104·5 [29]; 104·6—106·3 [46]	98—109
	,,	7-10 ,,	104·0—106·3 [82]	104·0—104·5 [23]; 104·6—105·7 [36]; 105·8—106·3 [23]	90—105
Fowls	February	7-10 A. M.	106·0—107·7 [87]	106·0—106·5 [20]; 106·6—107·1 [39]; 107·2—107·7 [28]	63—73·9
	,,	1-4 P. M.	105·8—107·5 [85]	105·8—106·3 [22]; 106·4—106·9 [41]; 107·0—107·5 [22]	74—76·5
	,,	7-10 ,,	104·8—106·5 [78]		73·5—75·5
	May	7-10 A. M.	107·0—108·7 [83]	107·0—107·5 [19]; 107·6—108·1 [29]; 108·2—108·7 [35]	92—95
	,,	1-4 P. M.	107·2—108·9 [80]	107·2—107·7 [32]; 107·8—108·9 [48]	95—99
	,,	7-10 ,,	106·0—107·7 [95]	106·0—106·5 [54]; 106·6—107·7 [41]	92—95

*—Includes some records of 11 A. M. also.

@— ,, ,, ,, ,, 12 noon ,,

two slight rises of 0·1 and 0·2°F. were recorded. Grant, Millar and Worden [1942] found that in cows the temperature of a faecal mass as it is passed is considerably below that of the rectal mucosa. It seems that the passage of this cooler mass over the mucosa temporarily lowers its temperature.

OBSERVATIONS

Mean temperatures at various times of the day at different seasons

The full data are too bulky for publication and are retained in the Record office of the Institute. Mean and range figures only are given in this paper.

Table I shows the mean daily body temperature, minimum and maximum body temperatures, range of air temperature and the number of observations taken, for buffaloes, cows, sheep, goats and fowls at Izatnagar for different times in different months. Similar figures are given in Table IA, in respect of sheep and hill-bulls at Mukteswar, in which hourly body temperatures are noted. Table IB is a supplementary table of body temperatures of buffaloes, cows and Hariana bullocks obtained from the records of another experiment. The figures have not been included in Table I because the times are different. Table IC is a frepuency distribution table of 744 temperature recordings of 38 buffaloes, these being intended as a check on the recordings of the smaller number of animals shown in Table I. Table II shows for the various times and months the body temperature ranges in which most of the observations fell, the percentage of the observations falling within the range, and the air temperature range

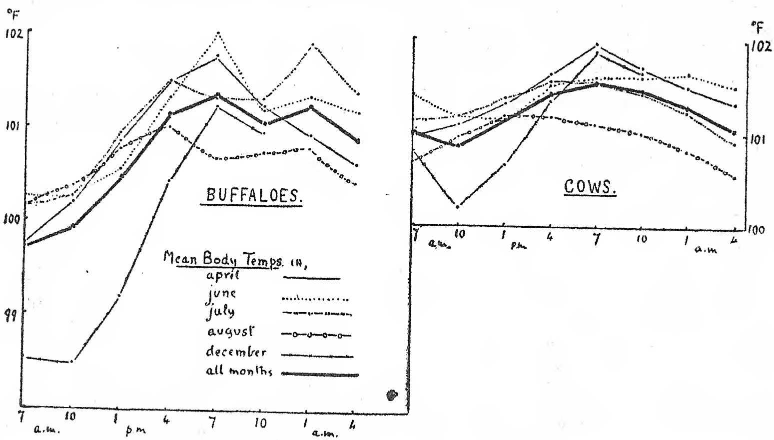

FIG. 1. Mean daily body temp. for different times

The data are also illustrated by charts. In Fig. 1 the mean daily body temperatures for different times during the month have been plotted, and in Fig. 1A the hourly mean temperatures of the sheep and hill-bulls are similarly displayed. From Fig. 1 it is seen that in buffaloes in December and April, the mean daily temperature is higher between 4 and 10 p.m. than at other times. In June, July and August the elevation is maintained to 1 or 4 o'clock in the morning. It is also seen

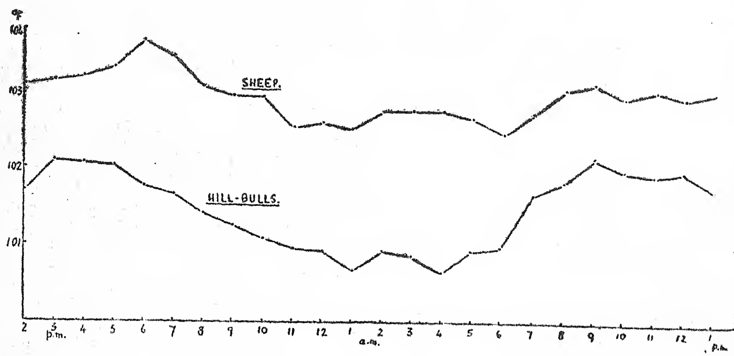

FIG. 1 A.—24 Hours recording

that the body temperature rises from 7 a.m. to a peak usually around 4 or 7 p.m. In cows the temperature usually rises from 7 a.m., in December only from 10 a.m., to a higher level, commonly around 7 p.m. The shape of the curve is, in general, flatter than that with buffaloes. The composite curves for all months clearly show that the temperature rises from 7 a.m. with buffaloes and from 10 a.m. with cows to reach a peak at 7 p.m. and then gradually falls. With sheep and goats (Fig. 3) the June temperatures were considerably higher than those of February. In both species temperatures showed a general tendency to rise from 7 a.m. and to reach a peak at about 7 p.m. Temperatures were thus higher from 4 to 10 p. m. than at other times. With fowls (Fig. 3), the mean temperatures for (end) May were consistently higher than those for February, but instead of rising after 4 p. m. they fell.

From the hourly observations made on hill-bulls and sheep at Mukteswar and shown in Fig. 1A, it is seen that the mean temperature starts to rise from about 6 a.m. and follows an upward trend till the end of the afternoon when it slowly falls to reach minimal values during the hours of darkness.

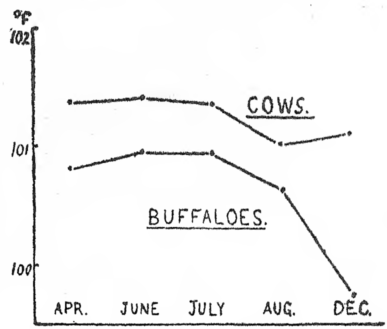

FIG. 2—Monthly variation in body temperatures.

For sheep, the average night reading (9 p. m. to 7 a. m.) was 102·72°F. (range 102·49-102·98) and day reading 103·15°F. (range 102·93-103·66). For hill-bulls, the average night reading was 101·0°F. (range 100·62-101·68) and day reading 101·88°F. (range 101·43-102·14).

Monthly variation in body temperature

Fig. 2 contains two composite graphs illustrating from mean daily readings the variations in body temperature which may occur from month to month. Similar

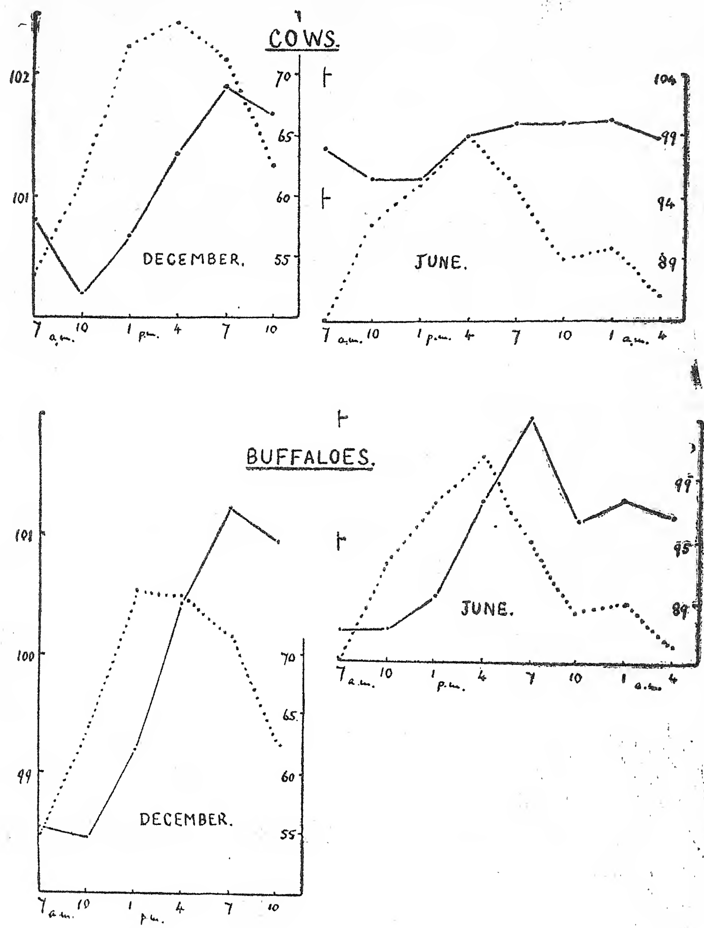

FIG. 3. Air temperature (on the right)..........
Body temperature (on the left)— . — . —

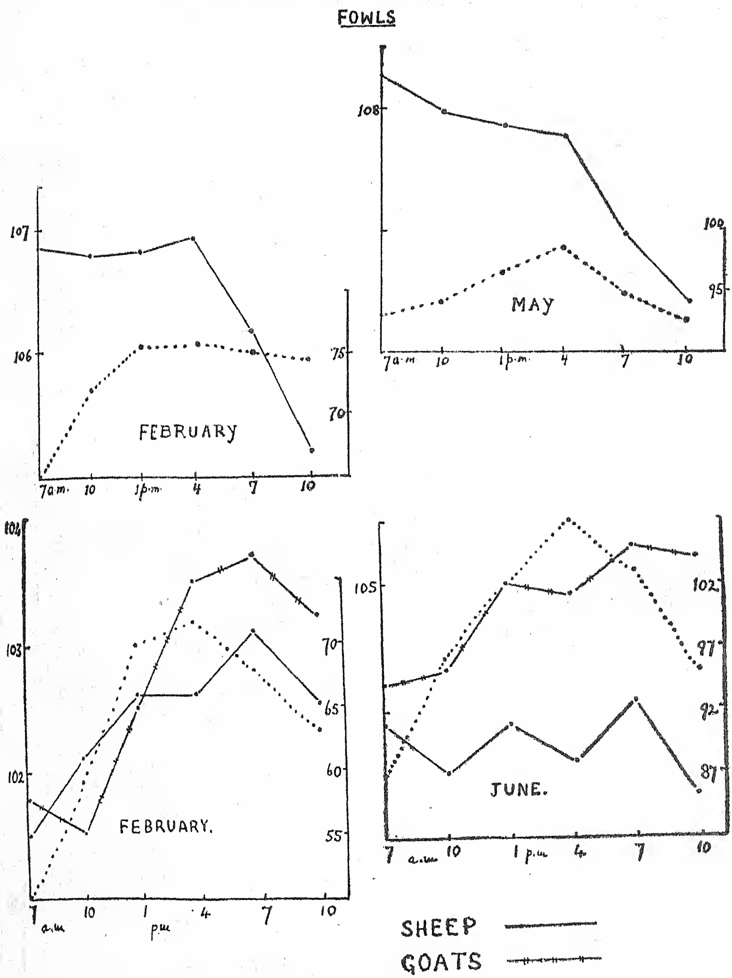
FOWLS
107
106
75
70
FEBRUARY
7a.m. 10 1p.m. 4 7 10
108
100
95
MAY
7a.m 10 1p.m. 4 7 10
104
103
102
70
65
60
55
FEBRUARY.
7 a.m 10 1 pm 4 7 10
105
102
97
92
87
JUNE.
7 a.m 10 1 p.m 4 7 10
SHEEP
GOATS

Fig 3 (contd.)

graphs prepared for particular times of the day are omitted to save space; however, the variations at particular times can be seen from Fig. 1. It appears that the body temperature shows no marked variation from April to July, in August it declines a little and still more in December in the case of buffaloes. The mean daily body temperatures in April, June, July, August and December, calculated from all the observations of the month as used in Table I, were,

for buffaloes: 100·81, 100·96, 100·96, 100·62, 99·74°F.

for cows: 101·38, 101·41, 101·35, 100·99, 101·09°F.

Thus between the winter and summer readings (December, June) the difference is 1·22°F. with buffaloes and 0·32°F. with cows.

Fig. 2 is not drawn for sheep, goats and fowls since the observations were made in two months only and the monthly variation can be seen by glancing at Fig 1. From all the observations it appears that the mean daily body temperature of sheep in February, at Izatnagar, was 102·41°F. and in June 103·77°F., difference 1·36°F. With goats the corresponding figures are 102·70°F. and 104·79°F., difference 2·09°F., [figures higher than those given by Sadiq [1943], especially during the hot season] and with fowls 106·46°F. (in February) and 107.53°F. (in May), difference 1·07°F.

Relation between air temperature and body temperature

The information just given on the body temperature variations within the day and at different months agrees with the now well-known fact that air temperature largely influences body temperature in the animal species considered. In Fig. 3 are drawn graphs of the mid-range air temperature at different times of the day for different months and the mean daily body temperature at corresponding hours. Without going into details, it will be seen that except with the fowls a rising or falling body temperature follows, after a lag period of perhaps three hours, a rising or falling air temperature. With fowls, there was no indication from these experiments that a rising air temperature influenced the body temperature, but a falling air temperature after 4 p.m. was coincident with a falling body temperature.

SUMMARY

The following statement shows the mean daily body temperature of various species of animals in different months, together with the air temperature range. For fuller information, the reader is referred to data tabulated in the paper.

Animals	Month	Air temperature (range, °F.)	Mean daily body temperature (°F.)
Buffalo, male	April ..	..	100·81
	June ..	81—108	100·96
	July ..	80—106	100·96
	August ..	73—102	100·62
	December ..	49— 80	99·74
Cow (Hariana)	April ..	..	101·38
	June ..	81—106	101·41
	July ..	81—105	101·35
	August ..	73—102	100·99
	December ..	48— 78	101·09
Sheep	February ..	48— 74	102·41
	June ..	80—109	103·77
Goat	February ..	48— 74	102·70
	June ..	80—109	104·79
Fowl, male	February ..	63— 76·5	106 46
	June ..	92— 99	107·53

2. Hourly observations over 24 hours on hill-bulls and sheep showed that the body temperature starts to rise about 6 a.m., follows an upward trend till early evening and then gradually falls to a minimum value at about 1 to 4 a.m.

3. In cattle and buffaloes, sheep and goats the body temperature level is influenced by the atmospheric temperature, variations in the latter being followed by similar variations of body temperature. The diurnal level therefore tends to be higher after 1 p.m. than before that time, the difference being far greater with buffaloes, sheep and goats than with cattle.

4. In temperature-taking, the personal factor is important. Without special instruction differences around 1·0°F. may be registered by different observers. The thermometer should be inserted as far as possible and left in position for one minute.

5. Following defaecation, the rectal temperature was lowered by 0·3°F. (mean) at 43 out of 51 trials.

AKCNOWLEDGEMENT

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A NOTE ON DERRIS DRESSING OF YOUNG CHICKS FOR THE CONTROL OF SEED-TICKS (LARVAE OF *ARGAS PERSICUS* OKEN)

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(Received for publication on 9 September 1944.)

Sen [1942] was successful in eradicating *Argas persicus* larvae from affected fowls by the application of a 10.0 per cent aqueous suspension of derris root powder. The treatment was tried by the authors on an adult fowl, a one month old and six two-week old heavily infested chicks in a private flock where 'seed-ticks' were causing annoyance and loss. Six of the chicks died within an hour of the derris application, while the two oldest birds suffered no apparent injury. In order to test the toxicity of derris applications, small-scale experiments were undertaken on chickens of different ages.

Experimental

The chicks used in these experiments were hatched and reared at the Institute poultry farm. In each experiment an equal number of untreated chicks were used as controls, all these remained healthy. Derris root powder was made into a 10.0 per cent suspension in water and after thorough stirring was applied with cotton-wool to the whole of body, except the natural orifices and eyes. The result is shown in Table I.

Table I

Mortality in chicks due to derris dressing

Age of chicks	Number dressed	Number dying after dressing	Remarks
2	11	7	Dead chicks showed acute parenchymatous nephritis. All deaths occurred within 18 hours of derris dressing.
16	11	7	
35	11	1	
60	11	Nil	

As derris dressing was found to be unsafe in very young chicks, and as Sen [1942] had applied the dressing on limited areas of the body, it was decided to experiment with partial dressing, using very young chicks (2 and 16 days old). The results are given in Table II.

Table II.

Result of partial dressing with derris

Age of chicks (days)	Dressing under wings		Dressing under wings and abdomen		Dressing under wings, abdomen and inside of thighs—	
	No. dressed	No. died	No. dressed	No. died	No. dressed	No. died
2	6	Nil	6	3	6	6
16	6	Nil	6	Nil	6	2

All deaths occurred within 24 hours of derris application, the dead chicks showing acute parenchymatous nephritis.

Summary

Derris root powder at 10.0 per cent watery suspension, as recommended for killing 'seed-ticks' (*Agras persicus*) on fowls, cannot be used for chicks up to five weeks of age owing to its toxicity. The toxic effects are in proportion to the extent of body surface covered. For adults the medicament is apparently safe and in these experiments 11 birds two-months old survived the application of the suspension over the whole body surface.

Acknowledgement

The writers wish to express their i tedness to Mr A. J. Macdonald, C In-Charge, Poultry Research Sect his interest and advice in this work

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SELECTED ARTICLE

VITAMIN A DEFICIENCY AND REQUIREMENTS OF FARM MAMMALS

By George H. Hart, Division of animal Husbandry, University of California, Davis

(Reprinted from *Nutrition Abstracts & Reviews*, Vol. 10, No. 2, October 1940)

Introduction

Vitamin A is essential for herbivorous animals but their food contains little or none. The riddle was not completely solved for nearly 20 years after MaCollum and Davis [1913] discovered the fat soluble vitamins, though it early became apparent that green plants also possessed vitamin A activity. Steenbock and his co-workers [1919; 1920; 1921] found a close correlation between the amount of yellow pigment in roots, maize, leaves and peas and their power to cure rats deprived of vitamin A. He offered the fruitful suggestion [1919] that vitamin A might be a leuco-form of a carotenoid pigment. Coward [1923], too, found that vitamin A activity was associated with the lipochromes in fruits and flowers and concluded that wherever carotene is found vitamin A may be expected to be present.

The significance of the observations of Steenbock and his co-workers, and of Coward was obvious, but they did not bear fruit for some years. The early attempt of Drummond [1919, 2] to ascertain whether pure carotene could be substituted for vitamin A miscarried, as carotene was unstable in the solvent, ethyl oleate, he happened to employ. Further, Steenbock, Sell and Buell [1921] could not discover any relationship between vitamin A potency and pigmentation in butter, cod liver oil or egg yolk.

In 1928, however, Euler, Euler and Hellstrom [1928] proved that carotene cured rats suffering from advanced vitamin A deficiency and believed that it was as effective as vitamin A. The matter was finally settled by Moore [1929] who showed that carotene was converted into vitamin A in the animal body and stored in the liver. The chemical relationship between vitamin A and carotene was believed by Karrer and co-workers [1931] to be such that two molecules of vitamin A were formed from the division of the symmetrical molecule of β-carotene with the addition of H_2O, this fission and hydrolysis presumably occurring in the body with the assistance of an enzyme.

In cattle, according to Moore [1932], only part of the carotene absorbed is changed into vitamin A, much being stored in the body fat. The proportions stored and converted vary with the breed of cattle and this accounts for differences in the depth of colour in their body fat and milk.

Preformed vitamin A has not been found in plant tissues but active carotenoids are present in all green plants, pumkins, sweet potatoes and carrots. Seeds, except yellow maize and palm kernels, are relatively free and the amounts present are inadequate for the nutrition of animals. Active carotenoids other than β-carotene which occur in plants are α- and γ-carotene and cryptoxanthin, the last being the biologically active pigment in yellow maize. According to Kuhn, Brockmann, Scheunert and Schieblich [1933] β-carotene has twice the biological activity of α- or γ-carotene, but this observation has never been confirmed, and the relative value of cryptoxanthin is also unknown. Karrer and Schlientz [1934] obtained principally β-carotene from grasses, spinach and nettles, and carotene from plant sources including alfalfa was found by Smith and Milner [1934] to have a high melting point and to be optically inactive, indicating that the β-form predominated. β-carotene is used as the international standard for vitamin A, and the vitamin A potency of the usual forage plants can, therefore, be deduced with reasonable accuracy from the amount

of carotene they contain, and expressed in international units of vitamin A.

Symptoms of vitamin A deficiency

Cattle

General

The effect of vitamin A deprivation has been extensively studied in the bovine. Although a variety of manifestations may be present the diagnosis, particularly in milder forms, may be difficult. Hart, Mead and Guilbert [1933] found that the time of manifestation varied in the same herd, because of differences in the previous food supply and amount of reserve stored by the animal. The state of maturity, lactation, gestation, development, production and rate of growth all influenced the onset of symptoms. Thus serious symptoms and fatalities may be present in young stock while adult animals show no symptoms. Newborn calves from dairies on 'alfalfa ranches' when taken to be nursed by depleted cows on a 'grain ranch' remained normal for 6 to 8 weeks. At the same time the calves of depleted cows on the same 'grain ranch' were weak and died in 1 to 5 days. This would indicate that newborn calves from the former farms had a reserve sufficient for a period of 6 to 8 weeks. Mead and Regan [1931], in their earlier work with calves on rations devoid of roughage, found that symptoms developed in 1 to 3 months after the animals were changed from whole milk and grain to a concentrate mixture low in vitamin A.

Halverson and Sherwood [1930] demonstrated that cottonseed poisoning of cattle was in fact caused by vitamin A deficiency. In one experiment a large percentage of steers on an exclusive ration of cottonseed meal and hulls developed symptoms in 88 days. In other experiments dairy heifers averaging less than 1 year of age were continued on the ration for 200 days before the advent of acute symptoms. Evidence is adduced by Flora *et al.* [1939] that animals on deficient rations containing cottonseed developed symptoms in a shorter time than when the rations did not contain cottonseed.

Guilbert and Hart [1934] studied the time required to deplete non-lactating cattle which had had ample opportunity to store vitamin A. Twelve steers 9 to 20 months of age were raised on native green pasture, irrigated alfalfa, or sudan grass, with alfalfa and sudan grass hay in the winter season. The first group of animals, 4 in number, 12 to 19 months of age, was killed at the start. Their livers contained from 500 to 830 blue units per g. of liver tissue. The second group, 6 in number, was placed on the experimental ration consisting of dried molasses beet pulp 70, rolled barley 14, cottonseed meal 15 and $CaCO_3$ 1 per cent. These animals were killed in pairs, 63, 121 and 282 days, respectively, after being placed on the ration; the last pair was in an advanced stage of vitamin A deficiency. The animals in this group showed a progressive loss of liver reserve. The first pair had 250 and 420 blue units (per g. of liver), respectively, the second, 160 and 50, and the last pair afforded only a trace of blue colour with the unsaponifiable matter from 20 g. of liver. Symptoms did not appear in the first 4 animals and were manifest after 225 days in the last pair. One after 252 days showed night blindness. The other animal had a slight nasal discharge on the 241st day and was also night blind on the 252nd day with some evidence of incoordinated movements. Facial paralysis developed in both animals and in one of them the left ear drooped. The appetite and general appearance remained normal. On the 276th day a mild diarrhoea developed; nasal discharge and muscular incoordination became rapidly more marked, the hair appeared rough, appetite failed, weight rapidly declined, but no lesions of the cornea developed. They were slaughtered on the 282nd day.

No abnormality was found on post mortem examination and the carcases passed meat inspection. Both animals had a dressing percentage of about 65. The fat was snow white, whereas those killed at the expiration of 63 and 121 days showed a creamy yellow fat, and those

illed at the beginning of the experiment howed a still deeper colour. Carotene ppears to be withdrawn from the adipose issue during vitamin A deprivation without coincident reduction of the reserve of at. The last pair showed a uniform daily increase of 1 and 1·3 lb. up to a few days before slaughter. This result is in harmony with that of experiments on swine by Lund [1938] in which vitamin A deficiency did not affect digestion, protein and mineral metabolism or the utilisation of energy, but the appetite was gradually lowered.

The third group, containing 2 animals, received, in addition to the concentrate mixture, 1 lb. daily of high quality field cured alfalfa hay. When slaughtered on the 127th day their livers had only 50 and 80 blue units, respectively, per g. of liver tissue, indicating that the limited intake of alfalfa had had little effect on the maintenance of their reserves.

In another experiment a heifer on a diet low in protein, phosphorus and vitamin A passed through a gestation and lactation but her milk flow was so meagre that her underdeveloped calf gained only 25 lb. in 5 months. Nevertheless the mother, after 14 months on the diet, showed no symptoms of vitamin A deficiency. Newborn calves were shown to have low reserves, indicating that the pregnant mother on high intake is unable to supply vitamin A to the foetus much faster than it is utilised. Dann [1932] showed this to be the case in rats and rabbits. Thus the foetus will suffer first when borderline deficiency is reached. In agreement with Drummond, Coward and Watson [1921] and others, colostrum was found to be relatively rich in vitamin A.

Eye. Bechdel, Honeywell and Dutcher [1928] reported blindness in 1 of 5 animals on a ration deficient in vitamin A. Eye lesions were reported in calves by Halverson and Sherwood [1930] in their studies on cottonseed poisoning. Meiga and Converse [1932] also reported the birth of blind calves in cows on low grade roughages. Mead and Regan [1931] demonstrated typical xerophthalmia in some of their calves raised on diets without roughage. Sanchez [1933] and also Schieblich [1934] described a similar condition in Spain. Two hundred milk cows taken to Seville from Switzerland gave birth to calves 80 per cent of which were blind without visible eye lesions. Native cows also gave birth to blind calves and the incidence decreased after green food was given.

In the severe outbreak reported by Hart and Guilbert [1933] the eye lesions in the animals from a few weeks to one year of age varied from profuse lachrymation and slight clouding of the cornea to extensive keratitis, ulceration of the cornea, loss of the aqueous humour, lens opacity and shrinking of the eyeball with complete and permanent blindness. Clouding and ulceration of the cornea was also present in the steers from 1 to 2 years of age. Practically all of the adult cows showed night blindness without corneal lesions.

Night blindness from vitamin A deficiency is believed to result from an inadequate rate of regeneration of visual purple. The nature of the defect has been elucidated by Wald [1934], and has recently been discussed and the pertinent literáture reviewed by Tansley [1939]. Cows that are deficient only during the early months of pregnancy may give birth to permanetly blind, but otherwise normal, calves. In young animals the amaurosis, once developed, is nearly always permanent, irrespective of the amount of vitamin A subsequently ingested. There has been some difference of opinion as to the cause of this form of blindness because it has occurred under conditions in which vitamin A deficiency was not suspected, and other possible causes have been suggested. The case reported by Crocker [1919] and considered to be the result of insidious rachitis were probably due to lack of vitamin A. De Schweinitz [1931], and De Schweinitz and De Long [1934] described a similar condition under the name of papilloedema or choked disc and suggested that the defect

might be hereditary. Moore, Huffman and Duncan [1935] described 24 cases of this condition in calves and growing dairy animals but concluded that it was different from blindness due to deficiency of vitamin A.

Hart and Guilbert [1937] experimentally produced cases of blindness in vitamin A deficiency. In these cases the pupils were widely dilated and there was a characteristic greenish colouration inside the eyeball when viewed through the cornea with the animal facing the light. There was pinching off and degeneration of the optic nerves where they pass through the optic canals in the sphenoid bone from stenosis of the walls of these canals. These authors regarded the bone changes as secondary, but, in view of the experiments of Mellanby [1938], this opinion may need revision. Kuhlman, Gallup and Weaver [1936] reported both xerophthalmia and the above permanent type of blindness in their calves on a diet low in vitamin A.

Mellanby [1938] produced deafness in young puppies by withholding vitamin A. There was nerve degeneration of the cochlear neurons and new bony growth in the modiolus, with degenerative alterations in the organ of Corti and the sensory epithelium of the semicircular canals. He regarded the overgrowth of bone of the labyrinthine capsule through pressure and stretching, to be the most probable cause of the nerve degeneration. New macroscopic bone formation was also found at the base of the skull. This author concluded that nipping in the various foramina was the cause of the degeneration found in the optic, trigeminal and facial nerves.

A report by Moore [1939], on blindness due to constriction of the optic nerve, papillary oedema and nyctalopia in calves deprived of carotene, has recently appeared. Papillary oedma was noted in 19 calves and 8 mature cows on low vitamin A rations, as well as in 3 calves at birth from dams receiving low intake of this factor. The night blindness was cured by the addition to the diet of carotene in the form of alfalfa hay. In none of these cases was xerophthalmia or keratitis observed. The blindness was attributed to intracranial pressure. It is presumed that permanent blindness occurs only in young animals because mature cows have the optic canal fully developed and calcified.

Mucous membranes

Vitamin A deficiency may affect epithelial structures in any part of the body as well as peripheral nerves and the central nervous system. Mason and Ellison [1935] suggest that vitamin A plays a part in protein metabolism within the epithelial cell. In deprivation the formation of glycoproteins or mucin seems to be retarded and that of albumenoids or keratin to be increased.

The respiratory tract is commonly affected and pneumonia is usually the terminal condition, causing death. This is frequently a subacute pneumonia with nodules the size of a pea or larger scattered through the hepatised areas and containing a mixture of air and pus cells. Lung abscess is another condition developing late in the deficiency. The legs are sometimes swollen and oedematous and this may be due to presence of parenchymatous nephritis.

HORSES

Vitamin A deficiency in horses has been reviewed by Edwards [1937] and the literature from widely scattered parts of the world cited. Mitchell [1930; 1931; 1933; 1935; 1936] considers roaring, stringhalt, navicular disease and bony exostoses to be manifestations of nutritional deficiencies, including deficiency of vitamin A as well as Ca and P imbalance.

In Finland Klemola [1933] showed that faulty hoops in the army horses were due to vitamin A deficiency. The scaly secretion of the peri-oplic band, which in places formed a thick spongy crust, changed to a normal, thin shiny, varnish like layer, when vitamin A or carotene was supplied.

The rations of horses as ordinarily made up in many parts of the world are

liable to be low in vitamin A. Particularly is this the case with army horses to which pasturage is not available. Meadows [1919] was undoubtedly describing this condition as occurring in horses and camels in Persia during the Great War and rendering them useless and dangerous for night operations.

Guilbert, Howell and Hart [1940] have determined minimum vitamin A and carotene requirements of some mammals, including the horse. Night blindness developed on the rations commonly used.

The necessity for two standards for expressing requirements, one for vitamin A and one for carotene, was pointed out.

Pigs

In their early experiments Hart and McCollum [1914] showed that when wheat kernel was the only food supplied, pigs developed dragging of the hind quarters and general paralysis, with a peculiar deflection of the head. This did not occur when milk or egg yolk was added to the diet. Histopathological studies of the tissues of affected swine were reported two years later by Hart, Miller and McCollum [1916]. Marked alterations were found in the motor cells of the spinal cord. At the time, these were credited to the inherent toxicity of the wheat kernel but the possibility was raised of the limited vitamin A in the diet being responsible. The paralysis was later shown by Hughes, Lienhardt and Aubel [1929] to be caused by nerve degenerations in portions of the spinal cord, optic, sciatic and femoral nerves of affected swine.

Hale [1933] reported that a gilt whose diet was deficient in vitamin A farrowed 11 pigs without eyeballs. Elder [1935] described cases in which incoordination occurred under natural conditions with a stringhalt like gait and posterior paralysis. This incoordination in some cases caused lateral spinal curvature with a swaying gait and gave the impression that the hind quarters could not track the fore quarters but followed off to one side. Swine paralysis due to vitamin A deficiency was also reported by Dunlop [1935] on rations which had been recommended for general use in England.

In feeding experiments with 0·5 to 2 per cent commercial cod liver oil given in conjunction with a meal mixture commonly used in pig feeding practice, Foot *et al.* [1939] found that pigs made good progress while controls on the meal mixture alone failed to thrive. The symptoms shown by pigs receiving no cod liver oil included loss of appetite, cessation of growth, impairment of vision in daylight, abnormal gait, convulsive fits and nervous collapse. Pneumonia or inflammation of the intestines or both occurred in all of 7 pigs that died during fattening.

Sheep

Sheep on a diet deficient in vitamin A suffer from night blindness and in some cases have permanent defective vision in semidarkness, irrespective of subsequent vitamin A intake or storage. Such animals cannot be used for the study of the minimum requirements of vitamin A by means of the night blindness test.

Aged ewes, without the drain of reproduction or lactation, are able to remain a long time on rations deficient in vitamin A without showing symptoms. In 4 such animals at this station, 22 months elapsed before night blindness was manifested. They were killed in cachexia after 27 to 30 months. As in cattle, vitamin A was withdrawn from stores more rapidly during the first year than subsequently. Onset of symptoms following the development of night blindness was gradual. They were anorexia, loss of condition and muscular weakness. In the final stages 2 of the animals had partially clouded corneas but sight in daylight was never seriously impaired. Pneumonia, enteritis and parenchymatous lesions in the kidney were the outstanding post mortem findings.

Efforts have been made to trace the etiology of urinary calculi to vitamin A deficiency but so far without definite proof. Calculi are more common in sheep than in other farm species.

Effect on reproduction

After it became established that vitamin A was essential for normal reproduction in mammals, the study of the effects of its deficiency on reproduction in the bovine species was complicated by the widespread existence of infectious abortion.

The early work of Hart, McCollum, Steenbock and Humphrey [1911] on the effect on reproduction of rations restricted to a single plant, wheat, oats or maize, proved conclusively that failure of reproduction could be of nutritional origin. Their first experiments led them to suggest that toxic bodies carried in the rations or produced in the intestinal tract, and also poor mineral content of the diet, might afford a possible explanation. Further work, published in 1917 after the discovery of vitamin A, did not suggest that the absence of this vitamin was the cause. In 1920 additional work with the oat plant was published and again reproductive failure occurred; this was attributed to low Ca. Hart, Steenbock, Humphrey and Hulce [1924] presented new observations and fresh interpretations of the previous work from which they concluded that the wheat plant was deficient in both vitamin A and Ca. The addition of these substances made the wheat plant ration complete for reproduction, thus making it unnecessary to continue to assume the presence of a toxic factor.

Hadley and Hawn [1929] and Hart, Hadley and Humphrey [1932] demonstrated that a good ration did not increase resistance to infectious abortion nor did a poor one increase susceptibility. In their experience lack of vitamin A was an important cause of failure of reproduction. Incidentally they confirmed the observations of Golding, Soames and Zilva [1926] that cod liver oil given to cows on a high plane of nutrition was responsible for reducing the butterfat by more than 20 per cent.

Meigs and Converse [1932] reported effects on reproduction in dairy cows of rations containing low grade roughage. After fairly long periods on grain and U.S. No. 3 timothy hay the animals gave birth to premature dead or weak and blind calves. When a better grade of timothy hay was given the proportion of normal claves was larger and, when the roughage consisted of U.S. No. 1 alfalfa hay reproduction was quite satisfactory.

Hart and Guilbert [1933] studied vitamin A deficiency in a severe natural outbreak in a herd of 250 head of which 100 died. Reproductive failure was complete in 25 to 30 cows calving at the height of the deficiency; all the calves were born weak, had severe diarrhoea and died in 1 to 5 days. Less severe manifestations were reported by Hart, Guilbert and Goss [1932] under natural range conditions during prolonged dry seasons.

When expulsion of weak or dead offspring occurs, a differential diagnosis can be made between this condition and that of infectious abortion, if conditions permit agglutination tests with *Brucella abortus* antigen to be made. The Carr and Price colorimetric test can be applied to liver samples of the foetuses and, if no colour is obtained, vitamin A deficiency is to be considered. Both conditions may be involved, as indicated by the experiments reported by Haring and Traum [1937]. Infectious abortion attacks the cells of the maternal and foetal placenta. The same cells are affected in vitamin A deficiency. Mason [1935] has shown that in vitamin A deficiency foetal death is secondary to marked placental injury, whereas vitamin E deficiency affects the foetal tissue primarily.

Diarrhoea in the newborn due to vitamin A deficiency may be confused with white scours. The history of the cases and analysis of liver tissue will help in diagnosis. If due to the former it will be promptly cured with vitamin A therapy. Severe losses from white scours occur when intake of vitamin A is ample and its use as a therapeutic agent under these conditions is unavailing. A recent report on this subject has been made by Stewart

and McCallum[1938]. Moore and Hallman [1936] have suggested from their experimental evidence that white spotted kidney in calves may be the result of vitamin A deficiency.

Ability of bulls and of cows to breed is not necessarily lost even when they are completely night blind and have muscular incoordination. Some evidence of structural changes in the seminiferous tubules of bulls has been reported by Guilbert and Hart[1935]. No sperm were found in the testes of one of their experimental animals that died of the deficiency. A young bull became so depleted as to develop permanent blindness from optic nerve constriction. Later, when supplied with adequate vitamin A, this animal became an active breeder, its semen contained mobile sperm, and it sired normal offspring, indicating that cessation of spermatogenesis, which may occur at the height of the deficiency, is not permanent.

In another experiment of Hart and Miller[1937] 11 of 17 ewes which at breeding time were night blind conceived and 2 had twin foetuses. The depletion was continued in these 11 cases and all of the lambs were born dead or died within 24 hours. Thus it may be stated in general that ability to conceive is affected late in the deficiency but failure of gestation is a relatively early manifestation. In hogs there is some evidence of apparently reduced prominence of the testicles in deficient boars as compared with controls of the same age. Gestation also fails in this species and, according to Hughes [1934] and Hughes, Aubel and Lienhardt [1928], resorption of the foetuses is common.

Although carotene is the pigment causing the yellow colour in the corpus luteum this has probably no function in reproduction. In the heifers studied by Mead and Regan [1931], which had no roughage but were supplied with cod liver oil, the corpus luteum was found to be completely lacking in pigment. Other animals in the same group were going through normal reproduction.

Storage and depletion

While storage of vitamin A under average condititions is ample for reasonable periods of low intake there are many conditions in which knowledge of the minimum requirements is of practical importance. The reserves of vitamin A in the newborn are low. With a given intake the rate of storage depends on the degree of depletion and rate of growth, so that maximum storage occurs in adults of advanced age. The reserves in tissus other than liver, fat and blood serum may be considered negligible. Semb, Baumann and Steenbock [1934] found that the blood serum of cattle contained significant reserves. Guilbert and Hart [1935] found that in cattle with livers having 300 to 500 blue units per g. the lung and spleen extracts gave negative tests and the kidneys contained only 1 to 5 units per g. The reserves in the body fat of these animals varied from 7 to 33 per cent of the total. In normally fed animals the reserves in the liver were largely composed of vitamin A, whereas in the fat carotene predominated. The data of Guilbert and Hart [1935] showed a withdrawal from storage of 4 to 5 mg. daily or obout 9 to 11 γ per kg. liveweight, and that a daily allowance of carotene approximating to the minimum requirement, as defined below, did little to diminish the rate of loss. Thus the intake must be considerably in excess of minimum requirements to maintain existing reserves and still greater to provide for storage in depleted animals.

Guilbert and Hart found that about 10 kg. dry matter from fresh green alfalfa would furnish 3·5 to 5·0 g. carotene and this would be equivalent to the estimated maximum storage in a very fat aged cow that had had access to green feed in abundance throughout her life. In one case a depleted cow was given about 15 g. carotene in green alfalfa over a 13-day period. At the end of the period she showed a storage of approximately 400 mg. or 2·7 per cent of the amount ingested.

This constituted an average daily storage of 30 mg. in excess of requirements. There was a concentration of 500 blue units per g. of liver tissue which is comparable to the amount present in the liver of 1 to 2-year old cattle kept under optimum conditions. These results compare favourably with those obtained in the rat by Davies and Moore [1934]. In one of Guilbert and Hart's experiments it had been shown that animals receiving 1 lb. daily of a fairly green chopped alfalfa hay in addition to a basal ration low in vitamin A did not show clinical symptoms of deficiency after a period 7 months longer than was required to deplete similar animals on the basal ration alone, but as two of these animals secreted milk so deficient in vitamin A that their nursing calves developed night blindness their intake must have been below the minimum.

Minimum requirements of vitamin A

A study was also made by Guilbert and Hart of minimum requirements for cattle with the cure of night blindness as a test for sufficiency. Experiments were started when the animals exhibited complete night blindness in semi-darkness, and frequently other nervous symptoms in addition. Small amounts of chopped alfalfa hay or dehydrated alfalfa meal of known carotene content were supplied and the quantity increased at intervals until normal weight increases occurred and clinical symptoms were cured. After 3 years' work, data were accumulated making it evident that the minimum daily requirement of carotene as supplied by alfalfa lay between 26 and 33 γ per kg. liveweight. Hay containing carotene, cod liver oil and halibut liver oil given orally were equally effective. Corotene, dissolved in olive oil and injected subcutaneously was less effective. Data were presented on animals varying in size from the rat to the cow which showed that utilization was related to bodyweight rather than to energy requirements and that in mammals 20 to 30 γ per kg. bodyweight constituted the daily requirement. The observations indicated that minimum requirements for chickens and turkeys are higher than for mammals.

Further work by Guilbert, Miller and Hughes [1937] on minimum vitamin A and carotene requirements of cattle, sheep and swine showed this to be 6 to 8 γ vitamin A or 25 to 30 γ carotene daily per kg. bodyweight. The minimum requirements for swine were within the range found by Dunlop [1935]. Evidence was presented that, at low levels of intake, vitamin A and carotene approach, weight for weight, biological equivalence and that the ratio widens as the dosage is increased.

Goss and Guilbert [1939] have recently revaluated the vitamin A in the oil used in the above experiments by spectrophotometric measurement, using the extinction coefficient at 328 mμ of crystalline vitamin A as prepared by Holmes and Corbet [1937] as standard, and on this basis the values 6 to 8 γ vitamin A per kg. bodyweight become 4·6 to 6·1 γ per kg. Comparison of this oil with the U.S.P. reference oil permitted expression of these values as I.U., namely 18 to 27 I.U. per kg. bodyweight daily. The most recent observations of the same group of workers [Guilbert, Howell and Hart, 1940] have shown that the requirements of the horse can be calculated on a basis similar to that used for sheep, cattle and swine; the daily requirement per kg. bodyweight for horses was found to be about 5 γ vitamin A or 20 to 30 γ carotene. The table in which these results are summarized is reproduced below.

At least 5 to 10 times this minimum level is recommended in practice. Significant storage occurred within a few months with this dose, but the milk of cows was low in vitamin A potency. Fraps, Copeland and Treichler [1934] consider

Summary of data on minimum vitamin A and carotene requirements of various species

Species	Daily intake per kg. bodyweight			
	Vitamin A		Carotene	
	γ	I. U.	γ	I. U.
Cattle	5·1—6·4	21—27	26—33	43—55
Sheep	4·3—6·3	17—26	25—35	42—58
Swine	4·4—5·7	18—24	25—39	42—65
Horse	4·2—5·3	17—22	20—30	33—50
Rat	4·6—5·3[1]	—	15—20	25—33
Rat	3·8—4·6[2]	18—22	—	—

[1] Data based on cod liver oil that was used in cattle, sheep, swine and horse experiments.
[2] Data based on U.S.P. Reference cod liver oil.

that green growing pasture grasses are needed to maintain the production of butterfat high in vitamin A.

Loss of carotene (provitamin A) during preparation and storage of fodder

Carotene is easily oxidized in the presence of air and light and the rate of oxidation is accelerated by heat.

Hartman [1931] found in the natural curing processes of hay in sunlight that, with the bleaching of the chlorophyll, carotene is rapidly oxidized and the vitamin A value reduced markedly. When the leaves of plants dry up and become brown their vitamin A value is completely lost [Coward, 1925]. Wide variations in the vitamin A value of hay depending on the method of curing have been demonstrated by many workers. Russell [1929] showed that a sample of alfalfa hay artificially dried had as much as 7 times the vitamin A value of field cured samples. On the basis of this work, 7 to 10 per cent dehydrated alfalfa meal, notwithstanding its greater cost, was recommended as addition to poultry food in the absence of fresh greens.

Generally speaking, artificially dried hays are higher in carotene content than field cured; marked variations exist is both. Kisselbach and Anderson [1931] and Guilbert [1934] found in some casen no significant differnce. Hauge and Aitkenhead [1931] studied the question and concluded that enzymes were important causes of loss of the vitamin during curing. The possibility of using antioxidants in stabilizing the vitamins is worth considering if it can be done within economic limits.

Guilbert [1934] has outlined a modification of existing methods for the estimation of carotene in forage. He [1935] used this method in studies of factors affecting the carotene content of alfalfa hay and meal. Tests were made on the effect of vacuum drying for 3 hours at 100° C. Four fresh leaf samples showed 48·9, 52·0, 46·0 and 60·3 mg. per cent, respectively, while the corresponding vacuum dried samples contained 48·4, 51·3, 46·6 and 57·1 mg. per cent. In similar tests on the effect of autoclaving for 1 hour at 17 lb. pressure, the loss of carotene varied from 33 to 67·5 per cent. Samples subjected to sun drying lost as much as 69·5 per cent of the carotene content. A sample previously dried *in vacuo* exposed under glass to the sun at the same time lost 67·4 per cent of its carotene. Enzymes were shown to play a part in the destruction of this substance. In one test, where leaves were exposed at 38° C. over water and toluene for 24 hours and then vacuum dried, a loss of 49 per cent occurred. In a control vacuum dried sample there was no loss.

Environmental temperature during storage is important. Sherwood and Fraps [1932] demonstrated the loss of vitamin A potency in cattle foods during ordinary storage, whereas Russell, Taylor and Chichester [1934] found that, when stored *in vacuo* at 0+5°C., alfalfa samples sustained no loss of carotene. The latter workers were of the opinion that the rate of carotene loss in cured plants might be greater during the early days of storage than after a few months had elapsed. Fraps and Treichler [1933] indicate the reverse to be the case. Guilbert [1935] made tests on alfalfa stored at 3 different temperatures. Samples in stoppered

glass tubes or in heavy paper containers stored for 8 weeks in the dark at temperatures of—5 to 5°C. sustained no loss; storage for 8 weeks at room temperature of 20 to 30° C. resulted in a 30 per cent loss. After 9 days in the dark at 60° and 80° C. there was 62 and 87 per cent loss, respectively. He showed that over a 15-day period the rate of loss of carotene in alfalfa leaves was roughly doubled for each 10° rise in temperature. The loss in whole leaves was only slightly less than in finely ground leaves. The rate of loss in ovens at both 40° and 60° C. was greater during the first 3 to 6 days than subsequently.

In storage under practical conditions losses of carotene were serious during summer weather, the mean temperature being 70° F. or higher. After several months under such conditions artificially dehydrated meal might be much lower in carotene content than freshly prepared sun dried meal produced under favourable conditions.

Peterson, Hughes and Freeman [1937] reported a modification of the Guilbert method of carotene estimation in foodstuffs. The methods of carotene estimation have been examined by the American Association of Official Agricultural Chemists, and Munsey [1937; 1938] as Associate Referee made two reports on the subject. In 1939 the Report of Sub-Committee A on Recommendations of Referees [1939] recommended that the Peterson Hughes method for the estimation of carotene be adopted as tentative, and the spectrophotometer be used or the 0·1 per cent potassium dichromate reference standard.

Ensiling is an important method of preserving fresh forage. The usual method of ensiling results in protein breakdown and fermentation during which carotene is destroyed. Virtanen [1938] modified the method so as to maintain the acidity of the ensiled material below pH 4, and thereby prevent the destruction of carotene. The method under the name of the A.I.V. process has been widely used and is a practical success.

RELATION OF CAROTENOIDS IN THE FOOD TO THE COLOUR OF MILK AND BODY FAT

Palmer and co-workers [1922] found carotene was the principal pigment in blood serum and in the body and milk fat of cattle and in skin secretions of Guernsey cattle. The yellow colour in chicken tissues, including egg yolk, is mainly xanthophyll. Swine, sheep and goat tissues do not usually contain an appreciable amount of pigment and their body fat is white. The subject has been studied by Hirzel [1935] in experiments with rabbits. In this species, as shown by Willimott [1928], xanthophyll is the main pigment in the fat. Xanthophyll also occurs in the fat of some strains of sheep in Australia. In the pigmentation of the fat in both rabbits and sheep in California, Guilbert [1936] has shown the pigment to be xanthophyll.

The widespread prejudice against yellow fat in the beef trade has little to support it, except for the tendency of the colour to increase in intensity with age and thus to be a sign that the meat is from aged animals. As the carotene furnishes an important dietary essential it is mischievous to permit trade practices which result in deprecation of its presence. Carotene is present in greatest quantity in the carcases of grass fed animals which are often lacking in 'finish,' and is particularly marked in old dairy cows of the Channel Island breeds. Well 'finished' cattle which are largely fed on concentrates with small quantities of roughage have fat which is relatively white.

Thus colour has become carelessly used in the meat trade as an index of quality, but this attitude has barely extended to the housewife or ultimate consumer. The grading of beef based upon conformation, quality and smoothness of the carcase with the degree of finish or covering with fat, is sufficient to place all carcases in their proper class.

Buckley *et al.* [1930] reported carotenosis in bovine livers, found during meat inspection. There was parenchymatous

degeneration with engorgement of the liver, the excess of carotene producing an intense yellow or reddish colour. The cause of the condition was not determined.

Carotene has been supposed to have some relation to one of the commonly occurring off-flavour termed 'oxidized'. It was found by Guthrie, Roadhouse and Richardson [1931] to result from contact of the milk with Cu or Cu alloys. Kende [1932] studied the relation of the diet given to cows to its development. It occurred in winter, and green feed or fresh hay was shown to contain considerable amounts of reducing substances which tend to prevent oxidized flavour or lessen its intensity. Anderson [1936] and Anderson, Wilson and Hardenbergh [1937] have concluded that the substance responsible for the beneficial effect of green food, artificially dehydrated alfalfa and carrots in preventing 'oxidized' flavour was carotene. Whitnah, Martin and Beck [1937] showed that spontaneously occurring oxidized flavour could be prevented on high carotene rations. Beck, Whitnah and Martin [1939] found no relation between frequency of occurrence of oxidized flavour and the lecithin or vitamin A content of the milk but it was effectively prevented by giving as little as 206 mg. carotene per head daily to cows that had been constantly producing milk with this off-flavonr.

Brown, Vanlandingham and Weakley [1939] found that ascorbic acid given at the rate of 1 g. daily partly, and carotene given at the rate of 350 mg. daily greatly reduced the production of 'oxidized flavour' by contact with metals. They were of the opinion, however, that the spontaneous development of oxidized flavour was not due to the ration being low in carotene.

The literature contains many references on the vitamin A and disease but that subject is too large to be covered in this review although some references to it have been made.

Epithelial cells are widely distributed in the body. It is logical to consider that the keratinisation of these cells would lower their resistance to invasion by bacteria and parasites. Many of the lesions produced in vitamin A deficiency result from secondary bacterial invasion and, in advanced cases of such deficiency these lesions overshadow the primary deficiency and are the apparent cause of death.

The claims of enthusiasts for the prophylactic action of vitamin A against infections have not been substantiated by experimental and clinical data. Nevertheless it is a safe maxim that to supply adequately the requirements of vitamin A in the rations of our animal populations is a part of good husbandry. It takesi ts place with many other factors in maintenance of better health, well being and production.

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ABSTRACTS

Research in nutrition: Its contribution to livestock production. GEORGE H. HART (1941). *Amer J. vet. Res.* 2, 131-35

IN view of the importance to the veterinary profession of new discoveries and constantly developing knowledge in nutrition, a brief review of development has been traced from the close of the last century.

Nutritional requirements vary with different species of farm animals. Contributions of recent researches reveal some very interesting facts.

The various factors in the water soluble vitamin B-complex, including thiamin, riboflavin, panthothenic acid, nicotinic acid and pyridoxine, have been proved to be synthesized by bacterial action in the rumen of cattle and sheep. It is therefore not an essential factor in the dietary constituents of these animals. This synthesis is also a probability in horses' caecum, but it is as yet unsupported by experimental data. Pigs require this B-complex in diet, as no such syntheses take place in their bodies. As the hatchability of eggs and viability of chicks are dependent on B-complex in diet, this also constitutes an essential factor in poultry breeding. Subcutaneous administration of vitamin C (ascorbic acid) has recently been shown to be of value in temporary sterility in bulls. Vitamin A is essential in all farm animals. The possibility of vitamin-Edeficiency is very doubtful, as vitamin E is widely distributed in foodstuffs or synthesized in the system of the domestic animals.

Animals living largely on roughages, as cattle and sheep, require bonemeal as a source of phosphorus in their diet. As bonemeal contains a high percentage of calcium, no extra calcium is needed when bonemeal is given. Hogs and poultry which get limited roughage require calcium in the form of ground limestone or oyster shell flour in their diet. Consideration in all cases has to be made of the cheapest source available of minerals supplied to livestock beyond what is contained in their regular feeds. Milk fever has been shown to be due to the temporary inability of the animal to transport calcium into the blood from the body store at the same rate as it is taken out in the milk of the heavy producers. Parathyroid as a pivot of calcium metabolism may be involved. Further research is however, necessary to sufficiently enlighten the point. Iodine gains its importance due to its deficiency appearing in widespread and localised areas. Iron deficiency is demonstrated in anaemia in young nursing pigs kept on wood or cement floors and is overcome by painting the udders of the nursing sow with iron preparations. In cattle combined deficiency of copper and iron, in which Hb content of RBC was greatly reduced, has been reported from Florida.

Heredity and environment are both important factors in the development of livestock. If the environmental level can be raised by improving the feed supply, then the limits of selective breeding can be proportionately extended. Without phosphorus supplement, attempts to improve the native cattle was a complete failure in western parts of the Union of South Africa. Environment in animals is largely a matter of management, mainly consisting of nutrition. Livestok production has definitely been benefited by nutritional researches, enabling the most economical use of the local cheap foods by success fully supplementing their deficiencies. Praric hay with calcium carbonate and grain mixture, for example, was nearly equal to alfalfa hay and grain mixture at much less cost.

It is, at present, possible to obtain a cheap and abundant supply of synthetic urea. It contains a high percentage of nitrogen and can successfully replace a part of the protein requirements in the diet of ruminants. The nitrogen is converted by bacterial action into protein. This discovery promises a successful application wherever protein feeds are costly as in the Hawaiian islands. [A. K. P.]

The pH of vaginal mucus of normal and sterile dairy cows. S F. SMITH and S.A. ASDELL (1941). *Amer. J. vet. Res.* 2, 167

THE authors examined the reaction of saline washing and of manually collected samples of the vaginal mucus of healthy cows. The pH values of the former were generally 0.5 to 1.0 lower than the latter. In tests on manually obtained mucus of both normal and sterile cows the pH was, with one exception (a cow suffering from pyometra), alkaline, although the pH dropped from about 8.0 to 7.0 for a short period at, or just after, oestrus. The mucus of the cervix was found to be more acid than that of the vagina. Injections of large doses of oestrogens did not cause the vaginal secretions to become acid and, on the whole, affected the vaginal pH but slightly.

In all these tests, the glass electrode was used in estimating pH. The authors conclude that sterility is not often due to an acid reaction in the vagina. [J. B. P.]

The determination and clinical correlation of variations in the calcium, inorganic phosphorus, and serum proteins of horse blood. A. HENRY CRAIGE, Jr. and JOHN. D. GADD. (1941). *Amer. J. vet. Res.* 2, 227-256

AFTER a general review of literature on the calcium, inorganic phosphorus and protein contents of normal horse blood as also the changes they undergo under variovs osteodystrophic disease conditions, the authors proceed to give their own findings and interpret the clinical variations with respect to various factors, such as age, sex, breed, season, pregnancy and diseased conditions, etc. They found hat the period allowed to elapse between the time of collection of blood and its chemical examination, had some effect on the inorganic phosphorus, calcium, and protein values. If, however, the serum is separated soon after the blood gets clotted, there is little change observed in these values. Sex difference has got no influence on chemical composition of blood. Mares in foal and those recently delivered showed distinct variations from the non-pregnant mares, specially in the serum protein values. In pregnant mares, the total protein was high and the albumin: lobulin (A:G) ratio was considerably low, averaging 0.8 as compared with 1.3, the average for males and non-pregnant females. With respect to calcium, there was a definite tendency towards hypocalcemia during the final stage of pregnancy when the fetus was making strong demands on the dam for calcium to promote ossification. Inorganic phosphorus showed little alterations.

Age definitely lowers the inorganic phosphorus of blood. For foals less than three months old, the average is 7·1 mg/100 c.c. This average receded with advancing age to a minimum of 3.1 mg/100 c.c. for horses over five years old. In the case of pregnant mares, the value is still less for the first half of the year which exceeds the minimum (3.1) again during the second half. Age does not appear to have any influence on the serum calcium values, average being 12.2 mg/100 c.c. In the case of total serum protein, foals less than three months old showed a significantly low average (5.1), while for animals above this age, the average was 6.1 gm./100 c. c. The A:G ratio is the highest in the youngest group (below three months:1.7) reaching a constant average value (1.3) for animals above six months through an intermediate value (1.5 for animals between three and six months).

Seasons, too, have their effects. The total serum protein showed a distinct gradual rise and fall from one season to another. In June and February the highest levlls were obtained. A similar trend was observed in the level of total calcium. From a high level in July, the average calcium value sinks in August to rise gradually to another high point in November, while the proteins remain relatively constant until October. The average inorganic phosphorus in five year old horses, both males and pregnant and non-pregnant females, revealed a constant rise from a minimum of 2.8 mg/100 c. c. in March to a peak of 3.9 in July. Thence it gradually receded to 3.3 by September. A further reduction was encountered only in winter. Feeding had also slight effect, especially on the calcium values.

Based on an idea that the variations in calcium and phosphorus values are to a significant extent the result of interactions among calcium, phosphorus and protein, the authors have suggested an equation, viz. $Ca^{++} + 0.4 P - Cage = Fv$, where Ca^{++} and phosphorus (inorganic) are expressed as mgs/100 c. c. of serum; Cage is a constant varying with the age of the horse (table given); and Fv, the result, is normally 0 and Ca^{++} has been calculated from the figures for total calcium and protein according to the formula of Mc Lean and Hastings (**1935 Amer. J. Med. Sci. 189, 601**) in order to coordinate the results of calcium, phosphorus and protein analyses run simultaneously as an aid in indentifying primary disturbances in the blood calcium and phosphorus.

Cases with acute bone lesions (e. g. acute arthritis, fracture) revealed a high incidence of blood calcium and phosphorus deficiency the latter showing a greater variation. In subacute cases (e.g. subacute periostitis and ostitis), however, these effects were progressively less pronounced. Chronic bone lesions (eg. ringbones, spavins and other oxostoses of chronic nature) were rarely associated with calcium and phosphorus deficits in the blood. From the study of clinical subjects with diseases of the soft tissues, a distinct tendency toward slight hypocalcemia was observed in respiratory cases (eg. bronchitis, pulmonary emphysema); and diarrhoea was associated with marked reduction in the blood levels of both calcium and phosphorus. Hypocalcemia, on the other hand, was never encountered in connection with symptoms of tetany, etc. [B. N. M]

Observation on the longevity of the liver fluke, Fasciola Gigantica, in cattle. JOSEPH E. ALICATA, AND LEONARD E, SWANSON, *Amer J. vet. Res.* 2., 417-18

THE authors carried out experiments on the longevity of liver fluke, *F. gigantica*, and concluded that these flukes are usually eliminated by the end of one year, but in certain cases they may persist for even three years and four months.

Five steers of weaning age were selected from an area known to be free from fluke infection in cattle. These steers were given 800 encysted metacercariae of *F. gigantica* in gelatine capsule. Faeces samples were examined for fluke eggs every three to six months. Eggs started to appear in the faeces from the third month and continued to pass in large numbers for 16 months. There after, a marked decrease was observed in the number of eggs passed. One animal slaughtered at this time had no mature flukes. By the end of the second year, two infected steers showed no evidence of fluke ova in their faeces. In the remaining two, a few ova persisted in their faeces till they were slaughtered, the first at 37 months and the second at 40 months. [S. K. C.]

Semen studies in the bull. R. W. DOUGHERTY and H.P. EWALT (1941). *Amer. J. vet. Res.* 2,418-26

IN this article the authors have described the preliminary results of their studies on bull semen. The routine semen examination included 645 samples obtained from 103 bulls which were mostly herd sires and experimental animals. Four hundred and seventy-eight samples were obtained with an artificial vagina and 158 from the penis of the bulls following dismounting after service; the rest were taken by rectal massage, from the ampuli of a slaughter house specimen and from the vagina of cows immediately after service. Artificial insemination work was also undertaken to correlate the laboratory findings of the semen studies with actual breeding efficiency. For this work, 7 herd sires and 75 dairy cows of three different breeds were used. An artificial vagina was used to

collect the semen samples from the bulls; in each case 1-2 c.c. of semen was used to inseminate the cows and laboratory examinatien was made with the remaining portion of the semen to evaluate its quality. The laboratory examination of semen included (i) total amount of ejaculate (ii) microscopic estimation of motility (iii) spermatozoa counts (iv) tabulation of the number and different kinds of abnormal spermatozoa (v) pH determination. Semen samples were evaluated as excellent, good, fair or poor, and the sperm counts were made with haemacytometers with normal saline as the diluting fluid. In making semen smears, ordinary blood smear method was used, which were, stained with eosinhaematoxylin method after being fixed in Schaudinns solution. Hydrogen-ion determinations were made with a potentiometer with glass and colomel electrodes.

The authors could not find any correlation between the quality of the semen samples as evaluated by the laboratory methods employed by them and the actual breeding performances. In one bull, however, a slight negative correlation was noted between the number of abnormal forms of spermatozoa and the fertilizing ability. The results obtained by the authors demonstrate a considerable diurnal and daily variations in the characteristics of the semem, differences being more pronounced in samples obtained from bulls in excessive sexual use. A correlation was noted between the motility and viability of spermatozoa and changes in the pH values of semen in the first few hours after the sample has been taken. Sperm motility has been found to be a factor in causing the hydrogen-ion changes of semen. Very motile samples showed a gradual rapid decline in pH. Semen samples of two bulls known to be of poor fertility were usually alkaline and on standing, the pH values increased. When the spermatozoa were inactivated by refrigeration or treatment with 0.2 c. c. of a 1:1,000 solution of bichloride of mercury, the rapid decline in pH was prevented. Hydrogen-ion determinations were also made on seminal vesicle secretions and ampuli contents of 17 abattoir specimens; pH of the former varied from 5. 59 to 6. 48 and that of the latter from 5. 66 to 6. 33. It has been suggested that pH deteminations of semen samples should be made immediately after collection.

A few preliminary experiments have also been made to find out whether or not there is any correlation between the ascorbic acid content of semen and breeding performances of the bull. Seventeen semen samples obtained from five bulls were analysed and the ascorbic acid content of semen varied from 0. 97 mg. to 8.08 mg. per 100 c. c. of semen. Although the ascorbic acid content of semen from one bull with poor breeding performance was rather high, the results obtained indicated a positive correlation between ascorbic acid content of the semen samples and the breeding efficiency of the bulls.

The authors remark that no single test can be used to judge the fertilizing quality of semen and that even when all the common tests are made the evaluations are of doubtful value except in extreme cases. [P. B.]

A method of determining the reproductive efficiency of cattle. H. E. KINGMAN and H. E. KINGMAN, (1942). J. *Amer. Vet. Res.* 3

IN this article the authors have shown how to keep and arrange records relating to reproduction in cattle so that the breeding histories of individuals or groups can be studied separately or collectively. They have discussed the importance of statistical analysis of data for determining the reproductive efficiency of the herd. Various methods of calculation of reproductive efficiency have been discussed and their merits shown. The following has been found to be the most suitable one.

$$\frac{\text{Cq (Calf equivalent)}}{\text{Cr (Cow years)}} \times 100 = \text{R (\%Reproductive Efficiency), where}$$

$$\text{Cq} = \frac{\text{M (Months of Normal Pregnancy)}}{\text{9 (Gestation period in cattle in months)}} \text{ and Cr} = \text{num-}$$

ber of cows in each year; the basis of calculation of a cow year is 12 months, but when a cow is in the herd for 9 months or more, she is included in the 12 month group. The general formula for estimating the breeding efficiency of the bull consists of dividing the number of successful services by the total number of services and multiplying by hundred, the result is expressed in percent. The methods of calculation for comparing the reproductive efficiency of a bull by various system of breeding or when comparing the reproductive efficiency of different bulls by the same method of breeding have also been given. The forms used at the Wyoming Hereford Ranch Trust for keeping the daily reports, records of individual cow compiled from the daily reports and veterinarians reports have been reproduced and their advantages discussed. The methods of analysis have been illustrated by charts, tables and graphs. [P. B.]

The bacterial content of goat milk. C. S. BRYAN, (1942). *Amer. J. vet. Res.* 3. 92-95

THIS study was undertaken to determine the incidence of infectious and non-infectious mastitis in dairy goats and the effect of udder infection on the quality and quantity of the milk produced. The tests employed were (1) standard plate count, (2) methylene blue reduction test and (3) resazurin reduction test. The chemical and cellular quality was noted by finding the percentage of chloride, the pH and leucocyte content of the milk.

In all the milk samples from 380 goats in 15 herds 2.3 per cent had streptococcal mastitis, 1.3 per cent had staphylococcal mastitis and 0.5 per cent had non-infectious mastitis; and 95. 9 per cent had neither infectious nor non-infectious mastitis. The distribution of samples among the various classes of the tests employed bears close relationship to the results obtained upon testing similar samples from cows; but the chloride determination, pH test and leucocyte count of milk cannot be used to determine the udder infection, although they accurately determine the chemical and cellular content of the milk.

The bacteriologic quality of the milk produced by goats, with and without udder infection, was determined by the methylene blue and resazurin reduction tests and the standard plate count; the results varied from one sample to the other.

Ninety-seven and one-half per cent of the non-infected, animals gave milk of class I methylene blue test quality0 94.0 per cent of class I resazurin test quality, and 4.g per cent had a bacteria count of over 1,000. Considerin the goats with staphylococcal mastitis, 56.0 per cen gave milk of class I methylene test quality, 40.0 per cent class I resazurin test quality, and 12. 0 per cent had a bacteria count of more than 1,000. The quality of milk produced by the goats affected with streptococcal mastitis was greatly reduced; only 40. 0 per cent gave milk of class I methylene blue test quality, 37. 0 per cent class I resazurin test quality, and 50. 0 per cent yielded milk with a bacteria count of more than 1,000. [R. H.]

Inheriarnce as a factor in poultry disease research. C. A. BRANDLY and N. F. WATEAS (1942). *Amer. J. vet. Res* 105-110

BIFFEN'S work on resistance to mycotic stem rust in wheat demonstrated the importance of inheritance in relation to diseases and laid the foundation of the present day knowledge of disease heredity. Progress with animals due to practical difficulties has, however, been much slower than in plants. Numerous examples are given of the inheritance of non-infectious pathological conditions in animals and birds. Reference is also made to the work of various workers upon differences in susceptibility among families within species or varieties. The role of hereditary resistance and susceptibility of various classes of animals to various neoplasms and leucemia has been definitely established by numerous workers. The possibility that a pathogenic organism or virus may change or mutate must also be borne in mind.

Stress is laid on the necessity of working on host material of known resistance or susceptibility. However, environment is also very important for the level of resistance which is inherited can be altered by feeding and management.

Though the fundamental principles of genetics are relatively simple, it is unfortunate that most of the economic characters in both plants and animals are dependant on the interaction between many genes and not on two genes as in some of the lower forms of life. Inbreeding automatically brings about homozygosity but at the same time it increases the diversity from family to family even though these families start from common stock. Inbreeding increases homozygosity for both good and bad characters but it does not create defects and it merely uncovers them by purification of the characters already present. Inbred strains of small animals have proved of great value in research but little systematic inbreeding has been done in large animals.

Little information is yet available about the mechanism of inherited immunity. In pullorum disease it has been shown that the lymphocytes are present in greater numbers and percentages in resistant chicks from the 18th day of incubation to the 3rd day after hatching.

Further resistance increases rapidly with age due to increase in the lymphocytes. Age resistance in chickens to *Ascaridia lineata* is due to an increase in goblet cells in the duodenal mucosa and the mucus secreted by these cells inhibits the development of the parasite.

Chickens and other species of poultry are very suitable for genetical studies in relation to diseases, for they can be kept fairly cheaply and large numbers can be bred rapidly. By exposure of unselected stock to standard infection, it is sometimes possible, even in one generation, to classify into resistant and susceptible groups.

In using the term resistance or immunity and susceptibility, it is important to remember that all of the individuals of any particular strain may not behave alike; some of the so-called resistant strain may contract the disease whilst others of the susceptible strain may not succumb. Statistical examination of all the data is, therefore, essential. Frequent testing of the host material is also essential if the degree of immunity is to be maintained or increased. [A. J. M.]

What research has accomplished in modern poultry production. E. JUNGHERR (1941). *Amer. J. vet. Res.* 2,259-61

THE author discusses the influence of research in building up the poultry industry and singles out four branches of poultry science, namely management, genetics, nutrition and pathology. The author does not give a critical review but merely cites various examples which have had much influence on the development of the industry.

Mass production of poultry has been rendered possible only through the introduction of artificial incubation, and amention is made of the pioneer work done by W. P. Hall and S. B. Smith. Research on house construction, ventilation and range management has also contributed to the growth of commercial poultry farming.

The remarkable achievement of a hen which laid 1,464 eggs in eight years at the Vineland contest is quoted as an example of what has been done through intelligent breeding based on critical research. The work of Raymond Pearl, who carried out the first systematic analysis on fecundity, along with the researches of Goodale and Hays on the factors governing egg production, is mentioned. Acknowledgments are made to work on progeny testing by Gowell and on sex linkage by Dunn, but no mention is made of the fundamental researches on sex-linkage by Punett and Pease.

In the field of nutrition, the work on poultry takes only second place to that on rats. Mention is made of the pioneer work by Eijkman who used poultry to investigate the anti-beri beri vitamin. Other contributions mentioned are the discovery of vitamin D by Mc Collum and Simmonds, the value of cod liver oil in preventing rickets in chickens by Hart Haplin and Steenbock and the value of manganese in preventing perosis by Wilgus, Norris and Henser.

In the realms of pathology, mention is made of the discovery of bacillary white diarrhoea by Rittger and the method of vaccination against laryngotracheitis introduced by Beauditte and Hudson.

The value of poultry meat and eggs in human nutrition is also touched upon. The discovery of vitamin K, the blood coagulating factor, in chicks and its present use in combating haemorrhage in man has proved of great value. The work of Rous and Goodpasture on chick embryos has proved of immense value in the production of human and animal vaccines.

Despite the advances that have been made, adult mortality has steadily increased during the last twenty years, both in America and Britain. Work on a large scale on the dreaded fowl paralysis or leucosis complex is now being carried out at the U. S. Regional Poultry Research Laboratories, East Lansing, Mich. The author calls for closer cooperation among all branches of research workers in order to meet the many problems still confronting the industry. [A. J. M.]

Co-ordinated trials with phenothiazine against nematodes in lambs. (Imperial Agricultural Bureaux Joint Publication 4,1943)

Conflicting reports on phenothiazine as an anthelmintic and the use of different criteria in the assessment of its efficacy led the Agricultural Research Council of the United Kingdom to plan and institute a series of co-ordinated trials at several centres in Great Britain to accumulate results that would admit of adequate statistical analysis, and would also furnish answers to certain specific questions. The present report is the outcome of findings at different centres.

The drug, having specific composition and character, was tested in single doses at varying dose levels against sheep nematodes, using egg counts, worm counts and lamb weights as criteria of efficacy.

Lambs, with a level of infestation of about 1,000 eggs per gramme of faeces were selected to represent the type that required treatment most. At each centre a unit flock of 28 lambs was used and given the following doses of phenothiazine in batches of four; 0 (controls) 5 gm., 10 gm., 20 gm., 30 gm., 40 gm. and 50 gm. In each batch two received the drug as compressed tablets and the other two as powder made into a drench with water. As a wetting and dispersing agent about 2 per cent sodium cetyl sulphate was used in both cases. In the control group, two received nothing, while the remaining two received sodium cetyl sulphate as a drench as was present in the 50 gm. dose, and they served as controls respectively against the lambs receiving the drug as tablets and as drench. Four unit flocks, kept inside for six weeks to avoid re-infestation, supplied information on egg and worm counts, and six others, maintained outside for sixteen weeks under natural conditions, provided data on the net effects of treatment on the growth of lambs. Each lamb was allotted to its flock (inside or outside), to its dosage level and to tablets or drench quite at random. Uniformity in respect of recording results was maintained at all centres.

Statistical analysis of the findings under different headings and other evidence revealed the following facts.

The main fall in the total egg count was obtained with the first 5 gm. of phenothiazine administered; beyond that level the diminution was comparatively small and irregular. The marked susceptibility of *Haemonchus* to a 5 gm. dose was mainly responsible for the diminution in egg counts, while larger doses were required for most of the intestinal worms to effect significant reduction. *Strongyloides*, *Trichuris*, *Nematodirus* and *Moniezia* gave anomalous responses, the last two actually showed increased counts with higher doses. Weight gains of lambs indicated progressive advantage with increasing dosage; the suggestion was that higher doses were responsible for the reduction not only of stomach worms, but also of certain intestinal worms, viz. *Bunostomum*, *Trichostrongylus* spp. and *Cooperia* considered to be of little importance in respect of lamb's health. It also appeared that weight responses were independent of the initial degree of infestation. The belief that heavily infested animals do not respond so well as lightly infested ones was not therefore substantiated. Tablets and drenches containing the same amount of phenothiazine did not differ in efficacy. It may be that drench is slightly more efficacious against stomach worms and, if so, it is possible that phenothiazine particles in a tablet require mixing with hosts bile salts before they are fully dispersed.

Full details are given of the statistical calculations and of the egg and worm counting techniques used. [S.S.]

Urea as a partial protein substitute in the feeding of dairy Cattle. E. C. OWEN, J. A. B. SMITH. and N. C. WRIGHT (1943). *Biochem. J.* 37, 44

UREA was fed to a group of seven lactating cows to determine its value as a partial substitute for blood meal. It provided up to 33 per cent of the nitrogen in the production ration or 25 per cent of the total nitrogen intake. For two cows urea was fed for a period of six weeks and then replaced by blood meal. For the other five cows, a period of about a fortnight, without either urea or blood meal, was inserted as a negative control period. Results for milk yield, milk composition, body weight changes, nitrogen balance, urea utilisation and the excretion of creatine and creatinine are given. Though urea is a diuretic agent, its feeding was reported to produce no excessive or harmful diuresis. Throughout the experiment, there was no marked change in the body weight of the animals. No definite conclusion is reported concerning the study of creatine and creatinine excretion. The nitrogen balance data show that the retention of urea was not complete. About 25 per cent of the ingested urea was wasted. Since a considerable individual variation was shown, the proportion of dietary urea, which was wasted, seems to depend upon the nutritional state of the animals prior to urea feeding. The urea content of the blood appears to rise at the start of the urea feeding and then adjusts itself to the normal value. The concentration of the non-protein nitrogen in the blood and that in the milk run parallel, as did that of the urea contents of the milk and the blood which never exceeded 28 mg. per 100 ml., a concentration which is considered harmless. In general the milk yields of the animals were well maintained when blood meal was replaced by urea and it produced no alteration on the percentage of protein, total solids, fat and lactose in the milk. [C. P. A.]

The use of enzyme-converted corn syrup in the manufacture of bulk sweetened condensed milk. P. H. TRACY and G., EDMAN *J. Dairy sci.* 1942, 25, 765

SUCROSS used in the manufacture of sweetened condensed skimmed and whole milk was replaced to the extent of 50, 75 and 100 per cent by an enzyme-converted corn syrup, containing 33.0 per cent of dextrose, 23.5 per cent of maltose, 6.4 per cent of higher sugars, 18.0 per cent of moisture, 18.3 per cent of dextrins and 0·3 per cent of ash. Samples containing corn syrup developed more colour during storage (11 weeks) than did all sucrose samples. The brown discolouration increased during storage, as the pre-heating temperature of the milk as well as the storage temperature was raised from 170 to 200°F. and 40 to 90°F. respectively. As for the development of discolouration, whole and skim milk behaved similarly. There was less physical thickening and less brown discolouration when the samples were stored at a lower temperature. Corn syrup lowered the pH of the condensed milks. Flavour changes during storage were least at 40°F, and greatest at 90°F.

Separate preheating of corn-syrup and milk to 185°F., replacement of 50 to 100 per cent of sucrose by corn-syrup and a storage temperature of 60°F. are recommended for the use of enzyme-converted corn-syrup in the manufacture of sweetened condensed milk of the U. S. A. standards of composition. [C. P. A.]

The synthesis and standardization of sodium resazurate for testing the hygienic quality of milk W. BAKER, J. G. DAVIS, W. G. LEEDS, P. OXLEY, W. F. SHOAT, R. S TWIGG and D. W. WATSON. *Biochem J.*, 36 Nos. 1-2, PROC BISCHEM. SOC. *S. PP.* i-ii. 1942

Two methods for the synthesis of resazurin are described; it may be synthesized by allowing nitric acid containing nitrous acid to act upon resorcinol in cold, dilute ethereal solution, or by the oxidation of an equi-molecular mixture of resorcinol and nitrosoresorcinol, with manga-

nese dioxide and sulphuric acid in a suitable solvent, such as aqueous acetone. The sodium salt is finally precipitated from strong sodium carbonate solution. Bacterial and tissue cells in milk reduce resazurin to resorufin, and ultimately to dihydroresorufin. The larger the bacterial and cell contents, the faster is the dye reduced. The test is carried out at a concentration of 1 in 200,000 in milk. The following standards for resazurin are suggested:—

(1) A content of 60 per cent $\pm$ 3 per cent of free resazurin.

(2) The content of resorufin should not exceed 3 per cent.

(3) It should give a water-clear solution on production in alkaline solution (0.05 per cent).

(4) At a concentration of 1:200,000 in fresh normal mixed Shorthorn milk of 3 to 4 per cent fat, a Tintometer disc reading of not less than 6 should be obtained.

(5) It should be free from any substances stimulating or inhibiting bacterial growth in the concentration used.

Resazurin is most easily purified by a repeated precipitation of the sodium salt from saturated sodium carbonate solution. [E. V. S.]

The Resazurin test for sterility of milk churns. J. G. DAVIS and D.W. WATSON. *Dairy Indust.* (8) 415, 1943.

THE method suggested by the authors consists in rinsing the churns with 500 ml. of sterile ¼ strength Ringer solution and adding one ml. of the rinse to 10 ml. of sterile separated milk in streile test tubes. The tubes are incubated at 22°C. for 24 hours. One ml. of 0.005 per cent resazurin solution is then added to the samples which are placed in a water bath at 37°C. Readings are taken in a comparator after 10, 30 and 60 minutes, using a tube of separated milk as a control. Any sample, reducing to a disc number 5 or less in 30 minutes, is regarded as unsatisfactory and has a count of more than 500 organisms per ml. of the rinse. Those samples, reducing to disc number 0 in 30 minutes, are regarded as very bad with a count of the order of 20-30 million per churn. The sterile separated milk to be used should be sterilized at 100°C. for one hour on three consecutive days and should be aged for at least one week but not more than three months. The plate counts of the churns are determined on yeastrel agar using ¼ strength Ringer solution for the dilution of the rinse. [E. V. S.]

Preparation and merits of churned cultured butter-milk. C. L. ROADHOUSE. 1942, *Milk. Pl Mon.* 31, 32.

THE author has described a method for making pleasing-flavoured, churned, cultured butter milk, containing butter granules and between 1 and 2 per cent of milk which does not readily 'whey off'. The method consists in pasteurizing the skim milk between 185° and 190°F. for one hour and pasteurizing the cream separately at 145°F. for 30 minutes. Then 0.75 per cent fat in the form of cream along with approximately 1 per cent starter is added, setting the milk at 70°F. and ripening to an acidity of 0.7 to 0.8 per cent before churning. Then it is churned at 68° to 72°F. by circulating the culture through a centrifugal pump and returning it to the vat. Salt is added at the rate of 2.2 ounces to each 100 pounds of milk. Then 0.025 per cent sweet cream is added, and finally the buttermilk is cooled to 40°F. or below. The butters may be coloured to whatever tint is desired. The butter granules remain more evenly distributed if the butter milk is not bottled until after it is stored for several hours. After storage, it should be agitated to distribute the butter granules before bottling. In order to retain the original flavour of churned cultured butter milk, the bottles should be surrounded by ice during delivery, and consumers should be advised to keep it cools. (E. V. S.)

Fly control in stables. Use of 'Gesarol' or the new 'DDT' in the control of stable flies. R. WIESMANN. *Soap* 19 No. 12 PP. 117, 119, 141, 143. New York, N. Y., 1943. (Translation of Eine Neue Methode der Bekampf ung der Fliegenplagen in stallen. Anz. Schadlingsk. 19 No. 1 PP. 5-8. Berlin, 1943)

THE principal disadvantage of the usual methods controlling flies in animal sheds in Switzerland is their lack of residual effect. Numerous large scale tests showed that various species of flies are killed in a short time by contact with deposits of Gesarol (a spray concentrate containing 5 per cent 22-bis (parachlorphenyl)—1,1, 1-trichlorethane, commonly known as DDT). In one experiment, which is described as an example of this, the bottoms and covers of petri dishes were sprayed with 1 per cent Gesarol and five freshly caught adults of *Musca domestica* L. were put into each dish after the deposits had completely dried. Flies were transfered to untreated dishes after exposure for 0·5, 1, 2, 5, 10, 20 and 60 minutes, and the effect was observed. There was none at the time of transference. Primary paralysis occurred 40 minutes after the shortest exposure and 10 minutes after the others. Secondary paralysis occurred in six hours, and 35, 35, 30-35, 30, 25-30, 20 and 20 minutes after the various exposures and death in 35, 8, 7-8, 6, 5, 3·4 and 3-4 hours, respectively. Results with *Stomoxys Calcitrans* L. were substantially the same time. The dry deposits on glass retain their potency for at least three months. The chemical acts as a neurotoxin, and the paralysis begins in the legs of the flies because the chemotactic sensorial organs in the tips of the tarsi come into direct contact with the deposit. In view of the results of the preliminary tests, the wall and ceilings of a shed containing ten cows and two heifers were thoroughly sprayed with I per cent Gesarol on 20 June 1942. There was a large manure pile near the shed in which *M. domestica* and *S. calcitrans* were breeding in enormous numbers, and thousands of flies of these two species could be counted on the walls and ceillings before spraying. The cows were very restless. While spraying was in progress, the flies dropped to the ground immediately and soon died. The shed was free from flies every morning until 10 July, 100-200 had entered by evening, but had been destroyed by morning. It remained practically free from flies until 25 July, although a slight decrease in effectiveness was becoming noticeable on 18th. The cows were quiet throughout this period and milk yield increased. On 1 August nearly 1,000 flies were counted in the shed and a second application of spray was made. Two applications, thus timed, will give protection for the whole fly season.

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REVIEW

Co-ordinated trials with phenothiazine against nematodes in lambs. *Imp. Agric. Bureaux, Joint Publication* 4, 1-56, 1943 Price. 3S. 6 d.

As an insecticide, phenothiazine has been in use since 1934. Harwood *et al* (1938) for the first time reported its efficacy against *Ascaris* and *Oesophagostomum* of pigs. Subsequently, numerous reports have been published on its use as an anthelmintic. In view of certain discrepancies in these reports and the fact that different workers used different criteria in assessing its efficacy, the Agricultural Research Council, U. K., initiated a series of planned trials, carried out by the same technique at several centres in Great Britain. These trials, involving some 280 lambs, were so planned as to yield results susceptible of adequate statistical analysis and afford data on the effect of phenothiazine, in single doses, against sheep nematodes, using lamb weights, egg and worm counts as criteria of efficacy.

The drug used fulfilled the specification suggested by Imperial Chemical Industries as desirable for phenothiazine for veterinary use. Only lambs with a level of infestation of about 1,000 eggs per gm. of faeces were selected. Each unit flock, besides reserves, consisted of 28 lambs and was given the following doses (in grams) of phenothiazine in batches of four: 0 (controls), 5, 10, 20, 30, 40 and 50. In each batch, except the controls, two lambs received the drug as compressed tablets and two as powder made into a drench with water. Two per cent sodium cetyl sulphate was used as a wetting and dispersing agent in both cases. Four of such unit flocks were maintained for six weeks inside, i.e. under conditions precluding reinfestation, and six unit flocks were kept for sixteen weeks outside, under natural conditions.

The observations recorded provide information on (I) the effect of the drug on particular species of worms as revealed by egg and worm counts of the inside flock, and (II) resulting net effect of the drug upon the lambs themselves, as shown by periodical weighings of lambs in the outside flock. The following points of interest emerge from these trials.

The greatest relative reduction in egg count was achieved by the lowest dose (5 gm.) used; higher doses reduced the counts further to a relatively small extent and irregularly.

From the worm counts, the stomach worms *Haemonchus* appeared to be markedly susceptible to lower levels (5 gm.) of phenothiazine. With the intestinal worms-*Bunostomum, Trichostrongylus* and *Cooperia*-the response was less marked but continued up to the highest dose used. Anomalous responses were obtained with *Strongyloides, Trichuris, Nematodirrus* and *Moniezia.*

In the outside flocks kept under natural conditions though the reductions in egg count were obliterated by reinfestation, there was a relatively large weight response which increased roughly in proportion to dosage up to the highest dose used. This must be due, judging from the 'inside' worm counts, to the reduction not merely of stomach worms but also of certain intestinal worms usually considered of little clinical importance.

No difference in efficacy was observed between tablets and drenches containing the same weight of phenothiazine.

The report contains a concise historical review of previous work on the anthelmintic efficacy of phenothiazine. The methods and techniques used are fully described and are satisfactory. A useful list of references is appended.

ORIGINAL ARTICLES

MULTIPLICATION OF *B. ANTHRACIS*, *CL. CHAUVOEI* AND *PASTEURELLA* IN ANIMAL CARCASES WITH A NOTE ON THE RATE OF COOLING OF CARCASES

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(Received for publication on 27 October 1944)

(With two text-figures)

A PROBLEM of importance in epizootiology, especially in hot countries, is the degree and duration of the infectivity of carcases of animals dead of disease. This is particularly so with such commonly occurring and acute diseases as anthrax, blackquarter, haemorrhagic septicaemia where the organisms are both numerous and widely distributed in the body of the animal at the time of death. In such cases not only has contamination of the immediate surroundings by body discharges to be considered, but there is also the more widespread distribution of infective material by carrion feeders. At the same time, there are a number of questions which should be answered if our knowledge is to be precise. We should have some idea not only of the extent of infectivity of the body at the time of death but also whether pathogenic bacteria, such as those of the diseases mentioned, actually multiply within the body after death particularly during the first few hours; if so to what extent, and what is the influence of environmental conditions such as atmospheric temperature. The effect of air temperature on rate of heat loss from bodies after death may be important in this connection and a note on this matter is included. With an organism producing early and sudden death in a highly susceptible animal, such as happens with a virulent strain of *Pasteurella* in a rabbit, cessation of multiplication of the organism would not be expected to coincide with the moment of death. With anthrax, the same may be true, unless the bacilli appear in the circulating blood mainly as a result of multiplication in internal organs. In the case of blackquarter, where at least some of the bacteria sporulate in the living animal, it is commonly believed that young vegetative organisms continue to multiply for some little time after death especially in the liver, and so further increase the number of spores available for infecting pasture land and water supplies.

To throw more precise light on such questions as these, some experiments were carried out with *Past. septica* in rabbits, *B. anthracis* in mice, guinea-pigs and goats, and *Cl. chauvoei* in guinea-pigs and sheep. In practice, it may be somewhat arduous to obtain the desired information, since it involves being present at the moment of death, which is frequently at night, in order to remove tissues for immediate use or for transfer to ice until they can be dealt with.

METHODS

Cultural

Bone-marrow was usually employed for estimating the variations in bacterial count which might occur in the carcases of animals dead of pasteurellosis, anthrax and blackquarter, since this tissue is easily obtained and can be made into a tolerably smooth suspension. Moreover, the marrow remains for a long time free from contaminating organisms. A femur was removed and the bone cautiously cracked with a hammer. With sheep and goats, the red marrow at the proximal end of the cavity was selected, 2 or 3 gm. being removed to a sterile tube for accurate weighing. In order to reduce the sampling error, approximately the same amount was taken in consecutive tests on the same animal. With rabbits and guinea-pigs, at first a similar technique was followed and as much marrow as possible (about 0·9 to 0·4 gm.) removed for weighing; latterly with guinea-pigs, weighing was dispensed with, as much marrow as possible being first removed and the remainder then collected by thorough washing with saline. After weighing, sterile sand was added, the mixture thoroughly ground and diluent gradually added with continued grinding up to a definite weight volume. From this, serial ten-fold dilutions were prepared by transferring 2·0 c.c. amounts to 18·0 c.c. diluent. In the case of pasteurellosis,

Ringer fluid was used as diluent, 1·0 c.c. amounts of each serial suspension being put into each of five plates, then eight drops of sterile heated ox serum and 10 c.c. agar at 48°C. With anthrax and blackquarter distilled water or saline was the diluent; with the former, three or five poured agar plates and with the latter five tubes of Robertson's meat-liver medium were sown from each serial dilution. In the early experiments a single tube of meat-liver medium was used for each dilution. (The value of this type of medium for supporting the growth of very small numbers of *Cl. chauvoei* has been shown by Haslam [1920]. The medium was always made up in the same way and usually by the same assistant. Liver broth containing about 2·0 per cent sheep serum, incubated for three days in an anaerobic jar, is also good, growth appearing at the bottom beneath the clear broth. The medium, however, is not quite so good and certainly not so convenient as the meat-liver medium.) When it was desired to know if or to what extent the organisms had sporulated, a portion of the first dilution from the original suspension was heated at 70°C. for 30 minutes.

Counts for *Pasteurella* were made after 48 hours at 37°C., deep colonies by that time being elliptical and whitish and surface ones circular smooth and with a bluish tint. Anthrax colonies were counted after 24 hours incubation by which time they are well-developed and of characteristic appearance. Both with *Pasteurella* and anthrax, plates showing some 100-400 colonies were selected when possible and the counts averaged. Wide discrepancies in counts on plates made from the same dilutions were uncommon and when present the experiment was rejected. The growth of *Cl. chauvoei* is indicated by gas production and a change in the colour of the meat particles from brown to reddish. Gas production appears to be a particularly reliable index. The final tubes showing growth were carefully noted after 48 and again after 72 hours incubation. Since exudates and suspensions of fresh bone marrow from sheep and goats tend to clot, the original dilution was usually made up in distilled water or saline containing 1·0 per cent sodium citrate. Every endeavour was made to follow the same technique in dealing with tissues removed from the same carcase owing to the necessity of getting comparable results, e.g. with muscular tissue from anthrax carcases the same muscle from the two hind limbs was used.

When tissues, other than bone marrow, or exudates were cultured, the procedure was the same in principle. In the case of muscle, fragments totalling 3·0 gm. in weight and as free as possible from macroscopically visible blood vessels were taken, finely minced in a mortar, very thoroughly ground with sand, distilled water added gradually up to 18 c.c. with continued grinding; after a few moments of settling the fluid was poured off, the residue again ground, further 9 c.c. water added as before and then mixed with the 18 c.c.

While the identification of *Pasteurella* and anthrax colonies presents no difficulty, this is not so with *Cl. chauvoei*. It is necessary therefore that organisms growing within the carcase and assumed to be those of blackquarter should be properly identified. This was done in many cases by cultural and immunity tests. For cultural recognition, blood agar slants were seeded in series starting with a loopful of meat-liver culture of the suspected organism, which was known to be free from aerobic contamination. The slants were incubated for 24 hours in an anaerobic jar and were then examined under a hand lens. Colonies of *Cl. chauvoei* are small and flattened, have entire edges and smooth surfaces and on the thinner parts of the slant can be seen to have haemolyzed the blood. For immunity tests, guinea-pigs were used which had received intramuscularly at about fortnightly intervals increasing numbers of blackquarter spores. After withstanding four or five such injections of spores, the guinea-pigs were able to support a subcutaneous injection of 0·3 c.c. meat-liver culture, 0·2 c.c. 5 per cent calcium chloride, mixed with saline up to 1·0 c.c. Three weeks or so after this injection the guinea-pigs are ready for use. (A safer method of immunization is to begin with formalinized meat-liver culture. To a paper-filtered three-day culture formalin at six parts per thousand is added and allowed to act for 24 hours at 37°C. 1·0 c.c. of the vaccine subcutaneously is followed 14 days later by a similar injection of 3·0 c.c.) For identification of a culture, one immune and one healthy guinea-pig are given subcutaneously meat-liver culture of the organism in question mixed with calcium chloride in the quantities just mentioned. If the culture is one of *Cl. chauvoei*, the healthy guinea-pig should die within two or three days with the characteristic lesions, while the immune should show nothing more than

a slight local reaction. Tests showed that meat-liver cultures of *Cl. welchii* and *Cl. septicum* were fatal in the above conditions to *Cl. chauvoei*-immune guinea-pigs.

Microscopical

With anthrax and blackquarter, in order to check the cultural results, films (standard loopful over one sq. cm. surface) and sections of similar thickness were prepared and stained by methylene blue for direct counting from tissues of animals immediately after death and again after the lapse of some hours. With blackquarter the films were from bone marrow (guinea-pigs) and the sections from liver (guinea-pigs and sheep). With anthrax, bone marrow films were unsuitable but satisfactory results were obtained with films from the heart-blood of mice, taken first from one side of the heart and then from the other. Between the first and second examination, the carcase was stitched up.

1. *Past. septica*

The strain in use was given to me by Mr V. R. Rajagopalan, and had been isolated from a bovine animal in 1939. By storage on blood agar under paraffin, it had retained a high virulence for the rabbit, 1·0 c.c. 24-hour broth culture at dilution of 10^{-4} subcutaneously killing 14-23 hours later. Death would occur with great suddenness, animals apparently well except for a little dullness, would fall on their sides, scream and die after a few struggles. The average number of *Pasteurella* colonies, developing from 1·0 c.c. bone-marrow suspension at dilution of 10^{-5} or 10^{-6} at various times after death is shown in Table I. The unopened carcases were stored in a room at temperature maintained between 60 and 70°F.

The results indicate that within six hours after death the organism multiplies in the bone marrow. The count then remains stationary up to 48 hours or so, after which the numbers usually start to decline. They are still numerous, however, and in pure culture in the marrow as long as eight days after death. At the period of maximal growth the mean number of living organisms per gm. bone marrow for 15 rabbits was 2181 millions (range 306-6840, S.D. 1866). In a buffalo calf dying less than 24 hours after inoculation with *Pasteurella* the count was 1467 millions per gm. of marrow.

Table I

Past. septica *in rabbit carcases*

Hours after death	Rabbit number														
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
	Average colony count from 1·0 c.c. 10^{-6}						Average colony count from 1·0 c.c. 10^{-5}								
0	47	27	22	466	..	..	..	..	..	..	..	..	..	..	..
6	397	1,209	312	1,307	..	..	..	..	..	..	..	..	..	..	..
8	..	..	..	..	..	..	485	1,063	263	190	..	..	..	..	..
9	..	..	..	..	..	..	..	..	..	..	328	134	253	472	759
18	..	..	..	..	581	364	..	..	..	..	..	..	..	..	..
24	..	..	..	..	..	..	537	1,056	..	..	..	..	..	..	..
39	..	..	..	..	..	..	..	..	..	..	202	..	..	..	..
46	..	..	..	..	..	..	..	..	165	217	..	..	..	..	..
57	..	..	..	..	..	..	..	..	..	..	..	127	..	..	..
81	..	..	..	..	..	..	..	..	..	..	..	..	90	..	..
120	..	..	..	..	..	..	..	..	..	..	..	..	..	119	..
168	..	..	..	..	6	..	..	..	..	..	..	..	..	..	..
192	..	..	..	..	..	133	..	..	..	..	..	..	..	..	..
194	..	..	..	..	..	..	..	..	..	..	..	..	..	..	198

2. *B. anthracis*

Cultural

Ten goats, mostly from other experiments, and 14 guinea-pigs were used. With the goats, the time of death after subcutaneous inoculation of approximately the same amount of culture was 31-85 hours (average 49). Within the hour after death one femur was removed and the marrow cultured at once, the carcase was then placed in a room at air temperature usually between 60 and 70°F. for 6-24 hours when marrow from the other femur was similarly cultured. In some cases muscle was examined. With some of the guinea-pigs the procedure was rather different; both hind legs were removed, one was put in the cold and the other at a higher temperature, usually 90°F. After 8 or 24 or more hours the marrow of each was cultured. In order to reduce the chance of haemorrhage at the time of amputation the operation was not performed for an hour or two after death and ligatures were first applied to the upper parts of the limbs. In all this work in spite of every care the results were erratic, most probably because the organism grows in threads and colonies do not develop from single organisms. It is, therefore, unnecessary to give detailed results.

Microscopical

Since cultural examination gave indecisive results, direct count was attempted of bacilli and chains in the heart-blood of mice and guinea-pigs immediately after death and after 6-12 hours storage of the carcase at 90°F. While in the case of mice dying early, viz. within 24 hours of inoculation, the chains had roughly doubled in length during six hours at 90°F.; similar evidence could not be obtained with guinea-pigs. The results as a whole indicate that multiplication of the anthrax bacillus after death is not a constant feature.

3. *Cl. chauvoei*

The guinea-pigs and sheep, used for the experiments, were inoculated intramuscularly in the thigh with spore suspension or meat-liver culture, with or without calcium chloride. In most cases the sheep carcases were left in a shed at prevailing air temperature, while guinea-pig carcases were stored at 28° or 30°C., the alimentary canal in some cases being removed immediately after death.

Cultural results

Preliminary experiments on 17 guinea-pigs indicated that:

(1) At the time of death, unless this is unduly delayed, the blackquarter organisms present in the blood, spleen and bone-marrow are in the vegetative form. In animals which had been dead some hours, e.g. during the previous night, spores might already have formed in the bone-marrow.

(2) At the time of death most of the blackquarter organisms present in the local exudate are in the form of spores, and cultures from the exudate are not infrequently contaminated with aerobes.

(3) In carcases kept for 24-72 hours at 30°C., blackquarter bacilli in blood, spleen and bone-marrow appeared to have multiplied and at least some of them had sporulated. One carcase was put into ice immediately after death and kept there for 44 hours when no multiplication was noted in the bone-marrow. A companion guinea-pig put at 28°C. showed multiplication in this tissue. Multiplication in the bone-marrow may proceed slowly up to at least the third day. The organism also sporulates readily at 30°C. in blood taken immediately after death and allowed to clot under vaseline. Lysis of the blood and gas production beneath the seal indicates multiplication [Hanna, 1897].

(4) In three of the animals it was proved by immunity tests that the organisms isolated from the blood after storage of the carcase were blackquarter bacilli.

Observations of a similar nature were made in 17 sheep. While in sheep, as in guinea-pigs, blackquarter organisms in the bone-marrow at the time of death are *usually* in the vegetative form, some of them may have undergone sporulation as early as three or four hours after death. In two sheep, vegetative organisms alone were present in the bone-marrow six hours after death, whereas in both cases 22 hours after death the organisms had sporulated. In sheep carcases left at the prevailing air temperature, multiplication of this organism followed by sporulation

appeared to take place in heart-blood clot and in bone-marrow. In many cases, meat-liver cultures grown from the *final* dilution of the series sown from tissues taken after storage of the carcase were identified as those of blackquarter. In the local lesion at the time of death, most of the organisms have already sporulated. If fresh exudate, to which citrate has been added, is incubated under a good vaseline seal for 48 hours at 28°C., multiplication of the blackquarter organism may at times be observed.

In the above experiments only one tube of meat-liver medium was inoculated from each serial dilution of the tissue but the results were consistent and indicated substantial increase in the number of organisms on storage. To give an idea of the richness in bacteria of tissues and fluids from animals dead of blackquarter, it may be said that growth in meat-liver medium was commonly obtained from amounts of material as small as the following :

Fresh heart-blood (guinea-pig) .	0·0002 to 0·00005 gm.
Fresh heart-blood (sheep) . .	0·0002 gm.
Fresh bone-marrow (sheep) . .	0·0002 to 0·00001 gm.
Fresh local lesion exudate (sheep) .	0·000002 gm.
Fresh muscle lesion (sheep) . .	0·0000001 gm.
Blood from guinea-pig heart after storage of carcase for 24 hours at 30°C.	0·000004 gm.
Bone-marrow from sheep after storage of carcase for 48 hours .	0·0000002 gm.

The results of a final series of experiments on bone-marrow from sheep carcases are shown in Table II. In these, five tubes of meat-liver medium were sown from each serial dilution of suspension covering the expected end point, 1·0 c.c. amounts being added to the culture tubes, and the probable numbers of organisms present in the original tissue suspension being computed from tables given by Halvorson and Ziegler [1933]. It appears that (1) the number of organisms present in the bone-marrow at the time of death varies considerably, (2) of the eleven cases—excluding sheep 318, the carcase of which was kept near freezing point—there was a quite definite increase in the numbers of organisms after death in eight cases, a slight increase in one and a reduction in two. In one of the latter (sheep 116), not only had the numbers fallen in the bone-marrow but no sporulation had occurred. In the sheep also, where slight increase only had occurred (No. 307), no sporulation had occurred in the bone-marrow. This sheep was killed when near death. Sheep 318 and 336 formed a pair, the carcase of the first being placed at 35°F. and the latter at 70°F. Air temperatures where not shown were probably around 60°F. (3) the increase though definite, varied greatly in extent. In five cases the increases were from 50- to 230-fold.

An estimate of the number of spores in the muscle lesion of a freshly-dead sheep gave a figure of 9 millions per gm., undoubtedly an underestimate. In one case a well-developed muscle 'tumour' and fluid exuding therefrom were collected from a freshly-dead sheep and found to weigh 1,254 gm. (muscle 697, exudate 557) a figure which will perhaps give some idea of the potential infectivity of such a carcase.

Microscopical

Multiplication of the organism can also be readily shown by direct count. With two guinea-pigs dead 24 hours after injection of culture mixed with calcium chloride, films of bone-marrow were made immediately after or within 15 minutes after death from one femur and similar films from the femur after the carcases had lain for six hours at 90°F. At the same time, pieces of liver were transferred to formalin for sections. With the films, the organisms present in 50 fields at random were counted and with the sections the numbers in 100 fields. The results were :

Guinea-pig 1 films . .	In the fresh tissue 0·4 organisms per field and in the stored tissue 7·16.
Guinea-pig 1 sections .	In the fresh tissue 0·003 organisms per field and in the stored tissue 4·98.
Guinea-pig 2 films . .	In the fresh tissue 0·78 organisms per field and in the stored tissue 8·1.
Guinea-pig 2 sections .	In the fresh tissue 0·072 organisms per field and in the stored tissue 15·72.

With a sheep, 15 minutes after death from blackquarter a piece of liver was removed to formalin and the carcase then stitched up and left for six hours at 70-80°F., when another piece of liver was removed. The counts (100 fields) were, in the fresh tissue, 0·52 organisms per field and in the stored tissue 9·86.

RATE OF COOLING OF CARCASES

I am unaware of any data on this subject in the case of animals. Since the rate of cooling may have a bearing on the multiplication of pathogenic as well as of putrefactive organisms in the body after death, some observations were made. Soon after the animal had expired, a small incision was made in the abdominal wall near the flank and

TABLE II

Cl. chauvoei *in bone-marrow of sheep*

Hours after death	Air temperature (°F.)											
	49-53	..	53-60	..	..	..	51-57	61-65	70	35	..	55-60
	Sheep number											
	183	100	307	143	74	17	199	84	336	318	228	116
0	2·31	6·31	4·93	7·0	7·0	17·1	94·3	141·0	27·2	240·0	..	..
6	..	..	..	1,090·0	..	918·0	..	..	..	..	329·0	..
8	..	..	..	..	..	..	..	..	..	..	..	240·0
30	..	..	..	..	..	..	..	..	..	..	109·0	4·56
38	..	..	..	..	..	..	..	..	700·0	240·0	..	..
45	..	..	..	..	..	..	171·0	..	..	..	..	..
48	542·1	329·0	7·29	..	1,090·0	..	..	..	..	..	..	..
50	..	..	..	..	..	..	..	70 0·0	..	..	..	..

Estimated numbers of organisms in 1·0 c.c. of the original tissue suspension are given in thousands. The original suspension was prepared at 1·0 gm. to 9·7 c.c. citrated saline, except in one case (sheep 17) where by mistake it was 1·0 gm. to 16·0 c.c.
Five meat-liver tubes were sown from each serial dilution of the suspension.

a minimum thermometer inserted so that its bulb lay on the surface of the liver near the diaphragm. In some cases the temperature in the depth of the muscles at the back of the thigh was also registered. At intervals from one hour onwards the thermometer was withdrawn until the top of the rider was just visible and then replaced. At the same time, the dry and wet-bulb temperatures of the air beside the carcase were recorded. Observations were made on rabbits, sheep, hill-bulls, a goat and a buffalo, as representing animals of different sizes, under varying atmospheric conditions. The results are shown in Table III and are also represented graphically in Figs. 1 and 2.

TABLE III

Temperatures (°F.) within carcases

Hours after death	Site	Rabbits		Sheep								Hill-bulls		Buffalo*	Goat*
		1	2	1	2	3	4*	5*	6	7	8	1	2*		
1	Liver	86·5	85	85	97	97	95·5	99	86·5	94	..	93	96	103	87·5
3	,,	78	79	82·5	95	92	93	97·5	85	90	..	88	94·5	102·5	82
6	,,	71	73	79	91	84	88·5	96	77	86	62	84	93·5	100	78
9	,,	..	..	..	..	..	..	95	..	..	57	..	92	..	75·5
12	,,	64	66	..	..	..	84	94·5	65	76	..	..	88·5	94	..
13	,,	..	..	..	..	..	..	..	..	..	..	..	..	..	73
14	,,	..	..	..	..	..	..	..	..	..	..	76·5	..	..	..
15	,,	63	62·5	..	..	..	..	..	..	..	..	..	..	..	..
18	,,	..	..	..	..	..	..	88	57	..	..	..	..	90	..
21	,,	..	..	..	..	..	..	..	..	70	..	..	..	..	..
23	,,	..	..	..	..	..	..	..	..	..	..	..	..	..	69
24	,,	..	..	71	..	..	78	90	..	..	..	71	86	95	..
25	,,	..	..	..	79	70	..	..	..	..	..	..	..	..	..
26	,,	..	..	..	..	..	..	..	..	67	..	..	..	..	..
27	,,	..	..	..	..	..	..	91	..	..	..	..	..	96	84
28	,,	..	..	..	..	..	..	..	40	..	..	..	..	..	97
29	,,	..	..	..	..	..	..	..	..	..	..	..	..	..	109
30	,,	..	..	..	..	..	77·5	..	..	..	..	69	..	..	114
32	,,	..	..	..	..	..	..	..	45	..	..	..	..	..	..
33	,,	..	..	..	..	..	..	..	..	..	..	..	..	..	100
34	,,	..	..	..	..	..	..	..	..	..	..	..	..	..	94
35	,,	..	..	..	..	..	..	..	..	..	..	..	..	..	89
36	,,	..	..	..	..	..	..	..	..	..	..	..	..	..	84
38	,,	..	..	..	..	..	..	..	42	64	..	..	..	..	..
39	,,	..	..	..	..	65	..	..	..	..	..	..	..	..	..
40	,,	..	..	67·5	..	..	..	..	..	..	..	..	..	..	..
42	,,	..	..	..	70	..	..	..	..	..	..	..	..	..	..
46	,,	..	..	..	..	..	..	..	..	..	..	64	..	..	..
48	,,	..	..	67	..	..	75·5	..	..	..	..	..	..	..	..
49	,,	..	..	..	..	64	..	..	..	..	..	..	..	..	..
50	,,	..	..	..	69·5	..	..	..	..	..	..	..	..	..	..
1	Muscle	..	..	84	91	95	..	..	..	..	..	93	..	..	..
3	,,	..	..	81	87	88	..	..	..	..	..	89·5	..	..	..
6	,,	..	..	76·5	83	86	..	..	..	..	..	85	..	..	..
14	,,	..	..	..	..	..	..	..	..	..	..	78	..	..	..
24	,,	..	..	71	..	..	..	..	..	..	..	71	..	..	..
25	,,	..	..	..	74	68·5	..	..	..	..	..	..	..	..	..
30	,,	..	..	..	..	..	..	..	..	..	..	68	..	..	..
39	,,	..	..	..	..	64	..	..	..	..	..	..	..	..	..
40	,,	..	..	67·5	..	..	..	..	..	..	..	..	..	..	..
42	,,	..	..	..	69	..	..	..	..	..	..	..	..	..	..
46	,,	..	..	..	..	..	..	..	..	..	..	63	..	..	..
48	,,	..	..	67	..	..	..	..	..	..	..	..	..	..	..
49	,,	..	..	..	..	63	..	..	..	..	..	..	..	..	..
50	,,	..	..	..	68·5	..	..	..	..	..	..	..	..	..	..

Rabbit 1 died of pasteurellosis 7.20 P.M. Aug. 12.
Rabbit 2 died of pasteurellosis 1.15 P.M. Aug. 13.
Sheep 1 wt. 33 lb. Died 4.30 P.M. July 27. Body wasted.
Sheep 2 wt. 45 lb. Died of rinderpest 3.10 P.M. Aug. 5.
Sheep 3 wt. 30 lb. Killed by stunning 8 A.M. Aug. 8. Body wasted.
Sheep 4 died at 12 noon on March 25.
Sheep 5 died of expl. B.Q. at 9.30 A.M. on May 22.
Sheep 6 died of expl. B.Q. at 5.30 A.M. Dec. 15. Immediately after carcase put on a verandah (air temp. 34-36°F.) six feet from falling snow.
Sheep 7 died of expl. B.Q. at 12.10 P.M. Dec. 15. Immediately after, carcase put in small room at 70°F.
Sheep 8 died of expl. B.Q. at 1 P.M. Dec. 16. Immediately after, carcase was laid on snow.
Hill-bull 1 chloroformed when moribund from pneumonia at 10-30 A.M. Sept. 12.
Hill-bull 2 died of rinderpest at 10-15 A.M. Apr. 16.
Buffalo died of rinderpest at 4-50 P.M. May 12.
Goat died at 10 A.M. March 30. Until the 23rd hour carcase was kept in a small godown since the sky was cloudy; from hours 23 to 30 there was sun, so carcase was placed outside on the ground, with projecting part of the minimum thermometer covered; body surface became very hot.
* observations at Izatnagar; otherwise at Mukteswar.

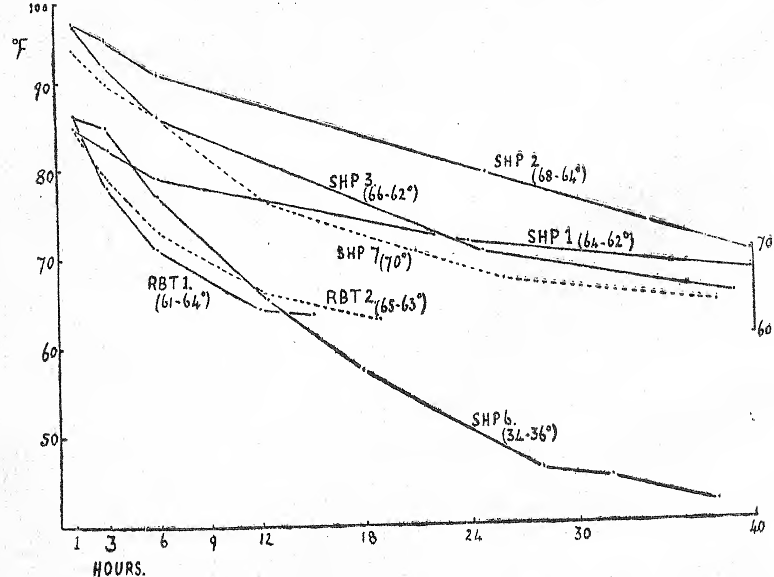

FIG. 1. Body temperature at liver surface: air temperature within brackets
BUF, Buffalow ; SHP, Sheep ; H. BUL, Hill-bull.

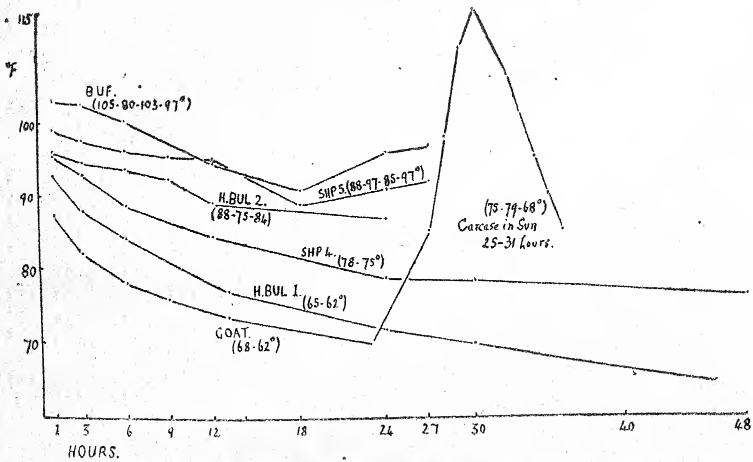

FIG. 2. Body temperature at liver surface: air temperature within brackets

Table III and Figs. 1 and 2 show the rate of decline of temperature under different conditions. It is seen that at 61-65°F. small carcases, viz. of rabbits take about 12 hours to cool to air temperature; at the same temperature larger carcases, viz. of goats and sheep take about 24 hours. At higher air temperatures, e.g. 88°F., they cool more slowly taking about 18 hours, while at temperatures not far above freezing point about 40 hours elapse. Cooling is always most rapid in the first six hours or so. The effect of air temperature is seen by comparing the hill-bulls in Fig. 2, and sheep 6 and 7 in Fig. 1. When the shade temperature rises after the body has cooled, the body temperature again starts to rise. Carcases exposed to the sun can become very hot (Fig. 2). The times stated are for a small number of carcases and, it must be emphasized, relate to a well-protected area in the centre of the body; peripheral parts will of course cool more quickly.

DISCUSSION

Multiplication of pathogenic organisms in carcases. Minett and Dhanda [1941] were unable to show that anthrax or blackquarter bacteria multiply in soil or in water. The extent of multiplication in the tissues after death was a matter for further enquiry. This subject has some special importance in India, where the warm climate favours retention of body heat in the freshly-dead animal. This clearly has the effect in the case of blackquarter of promoting bacterial multiplication and early sporulation. Since it must frequently happen that some hours elapse before carcases are disturbed by carrion feeders, larger numbers of bacteria (such as, *Clostridia*, *Pasteurella*) will be available for distribution than would otherwise be the case. The time of day at which death occurs will also obviously be of consequence. With anthrax, multiplication of bacilli in the carcase was not proved to occur, while on the other hand a higher temperature will presumably favour their more rapid disintegration. Likewise, Nunokawa [1910], although his work was mainly concerned with another problem, mentions that no substantial multiplication of the anthrax bacillus could be detected in the dead bodies of small laboratory animals at 37°C.

No data of a quantitative nature bearing on the subject have been seen in the literature, though some observations relating to blackquarter are available. In two papers by Foth [1909, 1910] blackquarter is considered, mainly from the standpoint of differential diagnosis from other diseases of cattle due to *Clostridia*. He reported incidental observations pointing to growth of the organism in the dead body. Thus with guinea-pigs smears of liver, spleen and kidney from the freshly-dead animal show only a few single rods. After the carcases have lain at room temperature for six hours they are more numerous. At the warmer time of the year there is an abundant increase, though not equally so in all organs and fluids. For this reason, blackquarter carcases undergo putrefaction more slowly, the apparent putrefaction being due to the gas-forming property of the blackquarter organism. In the liver and other organs and the blood the organism sporulates very quickly, especially in the warmer season. Warringsholz and Raszfeld [1924] give a good description of the post-mortem changes occurring in the liver of cattle in blackquarter. Shortly after death the organ is brown-red, blood-rich and soft; some hours later, the liver is dry and contains grey porous pea-sized foci. By 24 hours after death these foci are the size of walnuts or larger, have an ochre tint and a foam-like porous structure. Still later, the whole organ is foamy. Cohrs [1927], dealing with the histology of the liver of cattle dead of blackquarter, states that since the frothy condition of the liver is due to gas production by the organism, some multiplication in that organ must take place. He points out that temperature conditions in the slowly-cooling bodies of large animals are favourable for growth of the bacteria, especially in the liver and kidney which are well-protected. There can, therefore, be little doubt that in the case of blackquarter multiplication takes place after death and prior to sporulation, and the object of work reported in this paper has been to attempt some quantitative expression of the occurrence. No doubt, the results with sheep will apply *a fortiori* to cattle. One point concerns the duration of the illness. As pointed out by various observers, blackquarter is a septicaemic condition. Leclainche & Vallée [1900] state that, if animals die quickly from this disease, sporulation may not have occurred. This is certainly true of experimental blackquarter in sheep and guinea-pigs, so far as the blood is concerned. It may further be remarked that these animals often do not die suddenly but are moribund for several hours, a fact which probably influences the state

of the bacterium in the blood and internal organs.

It might be thought that early post-mortem invasion by putrefactive organisms would inhibit the growth of pathogenic organisms in the carcase. Thus, with guinea-pigs dead of blackquarter Leclainche & Vallée [1900] found that the organs were rapidly invaded by *Cl. septicum*. That has not been experienced in this work, where the only trouble was the occasional development in the tissues of *B. subtilis*. As regards anaerobic contamiuation, my experience agrees with that of Becker [1922] who found that guinea-pigs, killed from causes other than the injection of anaerobes and kept below 23°C. for less than 24 hours, show no anaerobic bacteria in the subcutaneous tissues, serous cavities or blood. Nevertheless, it is obviously necessary to make a proper identification of anaerobes found in carcases which have been stored in a warm atmosphere.

Body-cooling after death. A freshly-dead body may be likened to a katathermometer. In medical jurisprudence the rate of cooling gives a clue to the time of death and is stated to be almost proportional to the difference between the temperature of the body and that of the surrounding air. The rate in man is commonly put at 4°F., during the first three hours and 1°F. each hour afterwards. Brend [1934] gives 2° or 3° F. per hour for the first five hours, then 1°F. per hour. However, these are rough approximations because the rate of cooling is influenced by a number of factors, such as, (*a*) age—the bodies of middle-aged persons cool more slowly than those of infants and old people, (*b*) bodily condition—fat and well-nourished subjects retain heat longer than the lean and weakly, (*c*) manner of death—more rapid cooling in death from haemorrhage, starvation and chronic wasting than following fever or in sudden death of a previously healthy person, (*d*) surroundings of the body—space and temperature of air around the body, (*e*) body coverings, (*f*) surface on which the body is lying (bed, stone floor), (*g*) more rapid cooling in water than in air [Modi, 1920]. The period required for the internal temperature of a corpse to reach that of the air is given as 8-10 hours by Lyon [1928] for temperate countries, while Modi puts it at 15-20 hours. The period will tend to be shorter in India. In a general way the above facts will also be applicable to animals. From the few measurements made, it seems that at air temperatures between 60° and 70° F. small carcases lose heat by as much as 14°F. in the first hour and 3-3·5°F. per hour during the next five hours. With larger carcases (sheep) the rate of loss was found to be 4°F. in the first hour, 1·7-2·0°F. hourly in the next five hours and 0·8-1·1°F. hourly during the succeeding 18 hours.

Summary

1. In the case of *Pasteurella* and *Cl. chauvoei*, the organisms continue to multiply in the body for some hours after death. Some quantitative data to this effect are provided, based on cultural examination of tissues removed immediately after death and again after the carcase had been stored at air temperatures of 60-70°F. or above. The tissue mostly used was bone-marrow, since it can be easily manipulated. With blackquarter, the microscopical examination of such materials agrees with the cultural findings.

2. With blackquarter in sheep and guinea-pigs, at the time of death the organisms outside the lesion of inoculation are usually in the vegetative state. After some increase in numbers —of variable degree but sometimes 50- to 230-fold —they sporulate. If death is delayed, some may have already sporulated before death. The fresh local lesion contains enormous numbers of spores; a culture may develop from an amount estimated at 0·0000001 gm.

3. With acute pasteurellosis in rabbits the organisms continue to multiply in the bone-marrow during the first six hours, after which their numbers appear to remain stationary up to about 48 hours when they begin to decline. At the peak, the numbers may run to over 2,000 million per gm.

4. With anthrax, cultural results were erratic and there was no evidence that post-mortem multiplication is a constant feature.

5. Multiplication of pathogenic bacteria in the carcase is no doubt favoured by a warmer atmosphere, since body cooling will be retarded. Some data on the rate of cooling of carcases of different sizes are provided.

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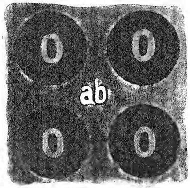

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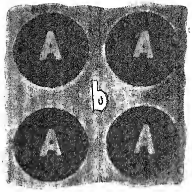

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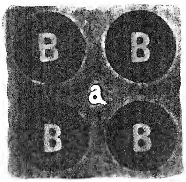

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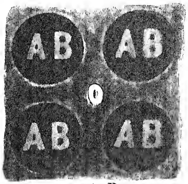

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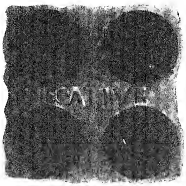

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THE BLOOD-GROUP IDENTIFICATIONS OF VARIOUS INDIAN BREEDS OF CATTLE IN INDIA

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(With Plate IV and two text-figures)

SINCE the inception of organized thought, it has been known that no two individuals are alike; hence have originated the systems of classification based on these differences. Morphological characteristics have formed the basis for classification into groups of similar animals or species, and in each species the differences of lesser degree, such as body contour and other qualities, have brought further subdivisions into breeds. The problem of the identification of various breeds of Indian cattle has recently been receiving much attention [Olver, 1938; Ware, 1939, 1941]. Ware recognizes 28 breeds of Indian cattle belonging to five main groups. This classification, as with other animals, has been carried out on the basis of morphological characteristics alone. The work detailed in this and a previous article [Singh, 1942] represents an attempt to approach the problem from another angle and to supplement the morphological findings by serological features with the special object of elucidating the origin and evolutionary relationship of the breeds.

The group classification of Indian cattle has been provisionally fixed after Little [1929] on an international basis with certain modifications, as follows:

Group 1 (International **O**). Serum agglutinative, cells not agglutinable; that is, cells with **O** isogen and **ab** isonin.

Group 2 (International **A** and **B** mixed). Serum agglutinative, cells agglutinable; that is cells with **A** isogen and **B** isonin or cells with **B** isogen and **A** isonin.

Group 3 (International **AB**). Serum not agglutinative, cells agglutinable; that is cells with isogen **O** or **AB** and serum without isonins.

Group 4 (Negative or N). Serum not agglutinative, cells not agglutinable. (In this group are included all cattle that have shown no reaction with the present method of testing.)

In the previous article [Singh, 1942] some account was given of attempts that have been made to define the blood-groups of domesticated animals. In this paper a few examples are given to show how by blood-grouping the origin of certain races of mankind may be discovered.

Parr [1929] provides data by which Armenians, Arabic Muslims and Arabic Christians can be assigned their racial indices. Goodner [1930] in tests on 223 pure Mayas and 202 Mayas with mixed Spanish blood in Mexico found practically all of the former to belong to group **O**, and in this way was able to differentiate pure Mayas from those of mixed blood. Shanklin [1935] found Rwala Arabs to have a high percentage of group **O**, and on this account he suggests that these Arabs are related to American Indians, who have a similar blood-grouping. Postmus [1934] found that type N was more frequent among the indigenous people in Netherland Indies than among Europeans. Edward *et al.* [1941] studied the racial distribution of blood-groups among Papags Indians in Anzona (U.S.A.). Out of 600 individuals examined, 93·8 per cent were of group **O** while only 6·17 per cent were of group **A** and groups **AB** and **B** were absent. The authors conclude that a high incidence of group **O** and the absence of groups **AB** and **B** suggest racial purity. Elsdon-

Dew [1939] in a blood grouping expedition in East Africa tested individuals of 46 tribes, and found groups **A** and **B** only. He interprets the distribution on the basis of mutation and suggests that the Bantu does not descend from the Negro but is rather a pure example of the common stock. An Egyptian origin is suggested for Hottentots. Thomas [1939] reports on blood-group distribution in England. Out of 5,000 people, 2,233 were of group **O**, 2,162 of **A**, 444 of **B** and 161 of **AB** group. Haldane [1940] studied the blood-group frequencies of 75 European population groups. A striking variation was discovered in the frequencies of **A** and **O** groups, among the peripheral population of Western Europe, there being a low proportion of group **B** in Scandinavia, Iceland, Spain, Portugal, Sardinia and the British Isles. Populations with low **B** are regarded as remnants of a primitive European civilization. In India, Malone and Laheri [1929] studied three types of people, (1) Turko-Iranians, (2) Indo-Aryans and (3) Dravidians. They confirm the findings of Hirschfeld in concluding that these people are characterized by a high incidence of group **B**. The Dravidians are also characterized by a high incidence of group **A**. Macfarlane [1938] studied the blood-group distribution in Bengal. Data from lower Bengal show that the frequency of groups **A** and **B** increases as one passes from higher to lower castes, the highest frequency of group **O** being found among caste Hindus. Bengalee depressed classes have the highest percentage of group **B** so far found in India. Bengalee-Muslims have a blood-group distribution similar to that of their low-class Hindu neighbours. Macfarlane [1940] also studied the problem in Baster State and found a high frequency of group **B** among the Bhils, this being regarded as due to in-breeding. Almost all the Chenchus of Baster State belonged to groups **O** and **A** and in this respect resemble the hill tribes of the Western Ghats and the Malyali lower castes, so sharing the distinction of being the only Indian tribe with more group **A** than **B**, Sarkar [1940], in 96 tests on certain primitive people of India, Huyani nagas (Assam and Bihar), showed the predominance of group **A** over **B**, excepting among the Thado-Kukis. Greval and Chandra [1940] tested Hindus, Muslims and Anglo-Indians of Calcutta. A high frequency of groups **O** and **B** was found in Hindus and Muslims, whereas group **A** was the highest and **B** the lowest among the Anglo-Indians. Sheshadhari-Nathan and Timothy [1942] carried out a similar investigation in Madras. Group **O** was the highest and **B** lowest among the Anglo-Indians. Pandit [1934] found a high frequency of group **B** among Todas (Guindy, Madras).

The technique used in the blood-grouping tests was the same as that described in the author's previous article [Singh, 1942]. It consisted of mixing in a small tube about four drops of serum and an equal amount of 2 per cent washed red cell suspension, gently mixing and exposing the mixture at 37°C. (dry incubator) for 30-60 minutes.

Cattle of the following breeds were selected for study: Kumauni (hill), Sindhi, Gir, Sahiwal, Tharparkar, Hariana, Kankrej, Amritmahal, Kangayam, Ongole, Dhanni, Afghan and Friesian. The distribution and location of these breeds can be seen in Fig. 1 from which it is evident that they are subject to a wide range of climatic and other environmental factors.

In this work there are three main sources of difficulty: (a) the low and quickly deteriorating titre of the serum. (This difficulty does not arise in blood-group work with human sera, which with cold storage can be used for some days), (b) the necessity of carrying out the tests at 37°C. and (c) the number of antigenic components in the erythrocytes of cattle. The location of some of the cattle in places where there are special transport difficulties was an additional hindrance. For these reasons the results recorded here are not as detailed as might be desired and the object has rather been to determine certain broad facts regarding the blood affinities.

The usual practice was to bleed 12 animals in the morning, test the 144 combinations of serum and cells, and read the results the same evening. The results largely depended on the particular antigenic components present in the blood of individuals of the group tested on one day. Occasionally, cells and serum from an animal found to be a good reactor on the previous day were included, in order to increase the chances of obtaining positive reactions. The reason for this is that a few cattle, giving no reactions with serum and cells from one lot of animals, may exhibit positive reactions with isogen or isonin* or with both these components when tested with serum and cells from another lot. In this way the group classification first recorded had to be altered after a further test, though this was not frequent.

* Isohaemagglutinins and Isofaemagglutinogens will be termed isonins and isogens, after Greval *et al* [1941].

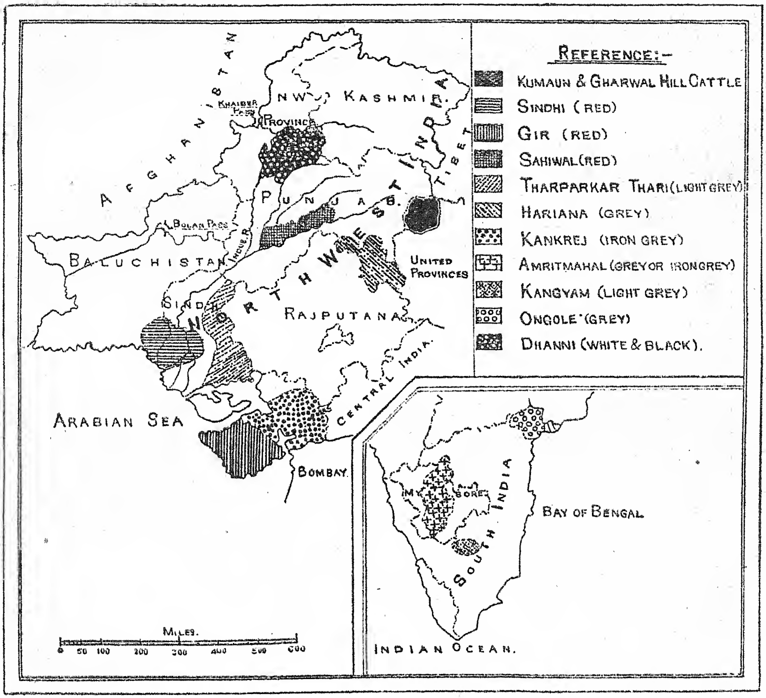

FIG. 1. Map showing the location of various cattle breeds in India

RESULTS

The results of the tests will be found in Tables I-V and in Fig. 2.

1. *Kumauni hill cattle.* The home of this breed is the Kumaun foot-hills of the Himalaya. It is one of the smallest breeds of Indian cattle, with short horns and a thoracic musculo-fatty hump. The poll and hump are usually covered with coarse hairs; the sheath is tight and the legs are short, thick and bony. The colour is generally deep-red or jet-black, and an admixture with white is regarded as indicating crossing with grey cattle of the plains.

The experiments were conducted at Mukteswar, where many pure Kumauni cattle are available. The animals were collected from various Kumauni hills and may therefore be regarded as of divergent strains.

In order to differentiate **A** and **B** groups, 24 of the reactors were selected and their isonins were cross-tested with their isogens. The results are given in Table I.

Table I

Blood-grouping of Kumaun hill cattle on 24 previously reacting bulls

	Cells from hill bull	SERUM FROM HILL BULL																								
		LOT I												LOT II												
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	Provisional grouping
Lot I	1	—	±	+	—	+	—	—	+	—	—	—	+	—	—	+	—	+	—	—	—	±	—	+	—	A & B
	2	+	—	+	—	+	±	+	+	—	—	—	—	—	—	+	—	+	—	—	±	+F	—	+	—	A & B
	3	±	—	—	—	+	—	±	+	—	—	—	—	—	—	+	—	±	—	—	—	—	—	±	—	A & B
	4	±	—	+	—	+	±	—	+	—	—	—	—	—	—	+	—	+	—	—	—	+	—	+	—	AB
	5	—	+F	+	—	—	—	—	—	—	—	—	—	—	—	—	—	+	—	—	—	+F	—	+	—	A & B
	6	—	+F	—	—	+	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	A & B
	7	—	+F	+	—	—	—	—	+	—	—	—	+	—	—	+	—	+	—	—	—	±	—	+	—	A & B
	8	—	+F	—	—	+	—	±	—	—	—	—	—	—	—	—	—	—	—	—	+	—	—	—	—	A & B
	9	+F	+F	+	—	+	+	+	+	—	—	—	—	—	—	+	—	+	—	—	+	—	—	+	—	AB
	10	+	+F	—	—	+	+	+	+	—	—	—	—	—	—	+	—	+	—	—	+	—	—	+	—	AB
	11	±	—	—	—	±	±	+	+	—	—	—	+	—	—	+	—	±	—	—	+	—	—	+	—	AB
	12	±	+F	—	—	+	+	+	+	—	—	—	—	—	—	+	—	±		—	+	—	—	+	—	A & B
Lot II	13	+	+F	—	—	+	+	+	+F	—	—	—	+F	—	—	+	—	+	—	—	+	—	—	+	—	AB
	14	+	+F	+	—	+	+F	+	+F	—	—	—	—	—	—	+	—	+	—	—	+	—	—	+	—	AB
	15	—	+F	—	—	+	—	—	—	—	—	—	—	—	—	—	—	—	—	—	+	—	—	—	—	A & B
	16	+	—	+	—	+	+	+	+	—	—	—	—	—	—	+	—	+	—	—	—	—	—	+	—	AB
	17	—	+F	—	—	+	—	—	+	—	—	—	+F	—	—	+	—	—	—	—	—	—	—	—	—	A & B
	18	+	+F	+	—	+	±	+	+	—	—	—	+	—	—	+	—	+	—	—	—	—	—	+	—	AB
	19	+	+F	+	—	+	+	+	+F	—	—	—	+	—	—	+F	—	+	—	—	±	—	—	+	—	AB
	20	—	—	+	—	+	—	—	—	—	—	—	—	—	—	—	—	+	—	—	—	—	—	+	—	A & B
	21	+	—	+	—	+	±	+	+	—	—	—	—	—	—	+F	—	+F	—	—	—	—	—	+	—	A & B
	22	+	—	+	—	+	±	+	+F	—	—	—	—	—	—	+	—	+	—	—	±	—	—	+	—	AB
	23	—	—	—	—	+	—	—	±	—	—	—	+F	—	—	±	—	—	—	—	—	—	—	—	—	A & B
	24	—	±	+	—	+	—	—	—	—	—	—	—	—	—	—	—	+	—	—	—	—	—	+	—	AB

Inoculation of isogen and isonin of lot I was done on 29 July 1942 (isogen isonin fresh).
Inoculation of isogen of lot I and isonin of lot II was done on 30 July 1942 (isogen 24 hour old in cold and isonin fresh).
Inoculation of isogen and isonin of lot I was done on 31 July 1942 (isogen isonin fresh).
Inoculation of isogen of lot II and isonin of lot I was done on 1 August 1942 (isogen 24 hour old in cold and isonin fresh).
The result of the tests was as before, except that with Nos. 15, 17 and 23, the grouping was previously recorded as O.

According to the reaction found in these tests, 13 animals were classified as groups **A** and **B** mixed. Of these, 12 animals (1, 2, 3, 5, 6, 7, 8, 12, 17, 20, 21 and 23) were then selected and their isonins and isogens cross-tested. The results, given in Table II, indicate eight animals belonging to **A** group and four to **B** group.

TABLE II

Analysis of bloods with **A** *and* **B** *mixed groupings selected from Table I*

(1) Isogen from hill bull	(2) Isonin from												(3) Grouping
	1	2	3	5	6	7	8	12	17	20	21	23	
1	—	±	+	+	—	—	+	+	+	—	+	+	**A**
2	+	—	+	+	±	+	+	—	+	±	+	+	**B**
3	±	—	—	+	—	±	+	—	±	—	—	±	$\mathbf{B_1}$
5	—	+	+	—	—	—	—	—	—	—	—	—	$\mathbf{A_1}$
6	—	+	—	+	—	—	—	—	—	—	—	—	**A**
7	—	+	+	—	—	—	+	+	+	—	±	+	**A**
8	—	+	—	+	—	+	—	—	—	±	—	—	$\mathbf{A_3}$
12	±	+	—	+	+	+	+	—	±	±	—	+	$\mathbf{B_1}$
17	—	+	—	+	—	—	+	[illegible]	—	—	—	—	$\mathbf{A_2}$
20	—	—	+	+	—	—	—	—	+	—	—	+	**A**
21	+	—	+	+	±	+	+	—	+	—	—	+	$\mathbf{B_2}$
23	—	—	—	+	—	—	±	+	—	—	—	—	$\mathbf{A_2}$

It is also noticed that the animals of both groups showed reactions against animals of their respective groups, thus suggesting the presence of sub-groups or serveral antigenic components of cattle cells. The problem was further studied by an absorption test conducted in the following way.

Equal quantities of packed cells from group **A** animals and serum from group **B**, and *vice versa,* were well mixed in a tube, left at room temperature for 30 minutes, then at 37°C. for an hour and finally at room temperature for 30 minutes. Since the serum titre was found to be very low, the amount of serum was subsequently increased to two, four and nine times the volume of packed cells. From this it was found that the optimal ratio for complete absorption of homologous isonins was 1 : 5. By absorption tests the following distribution of groups was found :

Groups	Hill bulls
A	1, 6, 7, 20.
$\mathbf{A_1}$	5.
$\mathbf{A_2}$	17, 23.
$\mathbf{A_3}$	8.
B	2.
$\mathbf{B_1}$	3, 12.
$\mathbf{B_2}$	21.

Thus in Kumauni hill cattle the ratio of groups **A** to **B** appears to be as 2 : 1.

2. *Red Sindhi.* The home of this breed is the province of Sind about Karachi, Hyderabad, the coastal line and the Indus river banks, including the Las Bela areas of Baluchistan. The animals are of red or fawn colour, frequently with some white on the face and dewlap. They are among the best milch type cattle of India and the bullocks are useful for draught. The animals are short-horned.

The tests were made in January and February, 1942 on 96 cattle (92 cows, 4 bulls) at the Imperial Dairy Institute, Bangalore, where a suitable herd of Sindhi cattle is maintained. It is a self-contained herd and the animals are therefore regarded as inbred for the purpose of this study. The results obtained with this and most of the other breeds are given in Table III.

3. *Gir.* The home of this breed is the Gir forest in Junagadh State (Kathiawar), where these cattle are found in a pure state. The breed is also spread over a large part of Bombay either pure or crossed with local cattle. The animals have short horns. The colour is generally mottled-red or black and of various shades of these colours. In some strains the body is mainly white with red or black spots, but entire reds and blacks are also met with. The cows are generally good milkers but the bulls are slow and lethargic in work. The Gir is reported to be the best beef animal in India.

The blood group investigations were also carried out at the Imperial Dairy Institute, Bangalore, where a small herd of these cattle is maintained and at the Palace Dairies of Bhavanagar and

TABLE III

Combined table of the 12 Indian and Afghan Breeds of Cattle

Serial No.	Name of breed	Grouping of Ware [1941]	No. of animals tested	O	Distribution of groups		Negative
					A & B Mixed	AB	
1	Kumauni (hill)	V	108	—	40 (37·04)	67 (62·04)	1 (0·92)
2	Sindhi (red)	III	96	36 (37·5)	7 (7·3)	47 (49·0)	6 (6·2)
3	Gir	III	120	50 (41·7)	5 (4·1)	50 (41·7)	15 (12·3)
4	Sahiwal	III	117	57 (48·7)	3 (2·6)	32 (27·4)	25 (21·3)
5	Tharparker	I	96	31 (32·3)	15 (15·6)	26 (27·1)	24 (25·0)
6	Hariana	II	132	16 (12·1)	4 (3·0)	34 (25·8)	78 (59·1)
7	Kankrej	I	120	27 (22·5)	3 (2·5)	25 (20·8)	65 (54·2)
8	Amritmahal	IV	96	18 (18·8)	9 (9·4)	43 (44·8)	26 (27·0)
9	Kangayam	IV	100	24 (24·0)	3 (3·0)	41 (41·0)	32 (32·2)
10	Ongole	II	96	18 (18·8)	2 (2·0)	24 (25·0)	52 (54·0)
11	Dhanni	—	99	36 (36·4)	8 (8·1)	36 (36·4)	19 (19·1)
12	Afghan	—	19	8 (42·1)	2 (10·5)	7 (36·9)	2 (10·5)
		Total	1,199	321	101	432	345
		Percentage		26·8	8·4	36·0	28·8

N.B. Figures in brackets indicate percentages.

Junagadh States. Most of the animals were farm-bred and may therefore be regarded as inbred for our purposes.

4. *Sahiwal.* These are essentially milch cattle and are generally raised in large numbers in the dry central and southern areas of the Punjab. They are long, deep, rather fleshy cattle, short on the leg, comparatively lethargic and heavily built, with fine skin, especially in heavy milking strains. The bullocks are not good for draught. They are short-horned. The colour is red but some are dun. Investigations were carried out at Jahangirabad Farm (Multan). The animals were mostly farm-bred and are therefore, regarded as inbred.

5. *Tharparker.* The home of these cattle is in the arid, semi-desert tracts of south-west Sind, but they are also bred in the adjoining Indian States of Kutch, Jodhpore and Jaisalmir. They are medium-sized animals, the cows have good milking capacity while the bullocks are good workers. They are lyre-horned and have straight limbs and hard feet. The colour is entire grey or light red in young bulls, while in cows and bullocks it is generally lighter grey. The colour tends to become white with age.

The investigations were conducted on a total of 96 animals at the Agricultural College Farm, Sakrand and at the Agricultural Farm, Lundo (Nawabshah Distt.), Sind. The animals in these farms had been recently purchased from various sources and may therefore, for the purpose of this study, be regarded as of divergent strain.

6. *Hariana.* The home of this breed is in the Rohtak, Hissar, Karnal and Gurgaon districts of the Punjab and in Delhi province. Hariana bullocks are powerful draught animals and are extremely useful for heavy ploughing and transport. The cows are fairly good milkers. They are short-horned cattle. The coat colour is white, a grey admixture being regarded as due to crossing with other breeds such as Kankrej.

The experiments were carried out at the Government Cattle Farm, Hissar, most of the animals

tested being farm-bred and therefore to be regarded as inbred animals.

7. *Kankrej*. This breed resides in the country south east of Kutch, extending from the south-east corner of the Tharparker districts of Sind to Ahmedabad in Bombay and from Deesa in the east to Radhanpur State in the west, particularly along the Banas and Saraswati rivers. They are fast and powerful draught cattle. In the past they have been exported to South America and other tropical countries in order to maintain the constitution of the local cattle by introduction of Zebu blood. The Kankrej has been said to be instrumental in the formation of beef breeds in these countries. They are lyre-horned cattle. Cows are fairly good milkers. The Kankrej is a thoroughly well-established breed which has been maintained pure for generations. They carry the head in a raised position, hence the name 'Proud Kankrej'. The colour is generally grey to iron grey, growing white with age.

The blood-grouping experiments were conducted at the Northcote Cattle Farm at Chharodi. Most of the animals were farm-bred and may be regarded as inbred cattle for the purpose of this study.

8. *Amritmahal*. This is a breed of Mysore State, where the animals are bred under Government control and their sale usually restricted. Subsistence is entirely by forest grazing. They are among the best and fastest draught cattle in India, and are particularly useful for army transport.

They have long and pointed horns arising close together and extending backwards and upwards. They belong to Ware's group IV. The colour varies from light to deep iron-grey, with a darker almost black shade over the shoulders and hind quarters in the case of bulls. Bullocks and especially older ones appear white, while the cows are generally white or at times light grey. Calves at birth are mostly red or light-red.

The tests were carried out at the Cattle-Breeding Station, Ajjampur, Mysore State.

9. *Kangayam*. This is a breed of the south-eastern taluks of Coimbatore, Madras. Animals of this breed are bred on scientific lines at the Livestock Research Station, Hosur. There is also a long-established herd maintained by a private breeder, the Pattagar of Palayakottai. The breed is suitable for light plough and transport work and is economical to maintain. The cows are generally poor milkers. The horns arise fairly close together and extend slightly backwards and upwards. The colour is generally light-grey but changes to white with age. Bulls sometimes show iron-grey patches over the front and hind quarters. Calves when born are red but later change to light-grey colour.

One hundred adults were tested at Hosur. Most of them were farm-bred and are thus inbred for the purpose of this work.

10. *Ongole*. The home of this breed is the Guntur district and neighbouring area of Madras. It is one of the large breeds of Indian cattle and greatly resembles the Hariana. The animals are, however, comparatively lethargic, and useful for heavy plough and transport work. The cows are fairly good milkers. They have short horns. In colour, they are mostly white but a small proportion are of a light grey colour. The bulls generally show a light shade of grey over the quarters. Ninety-six adults were tested at the Livestock Research Station, Guntur. Most of them were farm-bred and so inbred for our purposes.

11. *Dhanni*. This breed lives in the northern Punjab, in an area of undulating valleys at an average altitude of 1,500 feet. The climate is generally very cold during the winter and fairly hot in summer. It is a fast and light plough animal and fetches high prices. The cows are generally poor milkers. The horns are short. The coat colour of the body of the so-called pure Dhanni-bred animal is white with black spots, while the skin colour and tuft of the tail are invariably white. Ninety-nine animals were tested at Chakwal, Daulatala and Gujarkhan. As they had been collected from different sources, they may be regarded as of divergent strain.

12. *Friesian and Friesian-Sahiwal cross*. Tests on 86 animals were made at the Military Farms at Sialkot and Lahore. Some were pure-bred and recently imported from Europe, others were pure Friesians born in India. The remainder were crossed with Sahiwals. Results of the tests have been given in Table IV. The frequency of **A** and **B** groups in pure-bred Friesians is much higher than usual.

13. *Afghan*. The original home of this breed is Afghanistan. A few of these cattle were presented by the Afghan Government to H. E. the Viceroy and have since been kept at the Imperial Veterinary Research Institute, Mukteswar, where they have developed into a small herd. Nineteen cattle could be brought under experiment. The results are given in Table V.

Table IV

Grouping of Friesian and of Friesian-Sahiwal cross cattle

No. of experiments	Date	No. of animals	Group I (O)	Group II (A & B)	Group III (AB)	Group IV (Negative)	Remarks
1	11 April 1943	12	2	5	4	1	Friesian . . . . 18 Friesian-cum-Sahiwal . 1/2 . 3
2	12 April 1943	13	2	4	2	5	Do. . 3/4 . 39 Do. . 5/8 . 1
3	13 April 1943	12	..	3	4	5	Do. . 7/8 . 12 Do. . 11/16 . 3
4	14 April 1943	12	3	..	4	5	Do. . 13/16 . 7 Do. . 15/16 . 1
5	17 April 1943	12	1	4	6	1	Do. . 23/32 . 1 Do. . 53/64 . 1
6	17 April 1943	13	..	6	6	1	
7	18 April 1943	12	1	4	5	2	Total . . 86
	Total	86	9	26	31	20	
	Percentage		10·5	30·2	36	23·3	

Table V

Mukteswar—Blood-grouping of Afghan breed of cattle

Cells from	Age of animals (years)	Serum from																			Group distribution	Remarks
		1	2	3	4	5	6	7	9	10	2	15	11	18	17	8	12	16	1	14		
Afghan Cow 1	7	—	—	—	+	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	A & B	
,, ,, 2	$6\frac{1}{2}$	+	—	+	+	+	+	—	+	+	—	+	—	—	—	—	—	+	—	+	AB	
,, ,, 3	5	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	O	
,, ,, 4	5	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	O	
,, ,, 5	4	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	O	
,, ,, 6	5	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	O	
,, ,, 7	$5\frac{1}{2}$	+	—	+	+	+	+	—	+	+	—	+	—	—	—	—	—	—	—	+	AB	
,, ,, 9	6	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	O	Percentage :—
,, ,, 10	6	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	O	Group O . . 42·1%
Afghan Bull 2	7	+	—	+	+	+	+		+	—	—	+	—	—	—	—	—	—	—	+	AB	Group A & B . 10·5%
,, Heifer 15	$2\frac{1}{4}$	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	O	Group AB . . 36·9%
,, ,, 14	$2\frac{1}{4}$	+	—	+	+	—	—	—	—	—	—	+	—	—	—	—	—	+	—	+	AB	Group Negative . 10·5%
,, Bull-calf 18	$2\frac{1}{4}$	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	Negative	
,, ,, ,, 17	$2\frac{1}{4}$	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	,,	
,, Cow 8	4	+	—	+	+	—	—	—	—	—	—	+	—	—	—	—	—	+	—	+	AB	
,, Heifer 12	$2\frac{1}{4}$	+	—	—	+	—	—	—	—	—	—	+	—	—	—	—	—	+	—	+	AB	
,, Calf 16	$2\frac{1}{4}$	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	O	
,, Bull 1	$5\frac{1}{2}$	+	—	+	+	—	—	—	—	—	—	—	—	—	—	—	—	+	—	+	AB	
,, Heifer 11	$2\frac{1}{4}$	—	—	—	+	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	A & B	

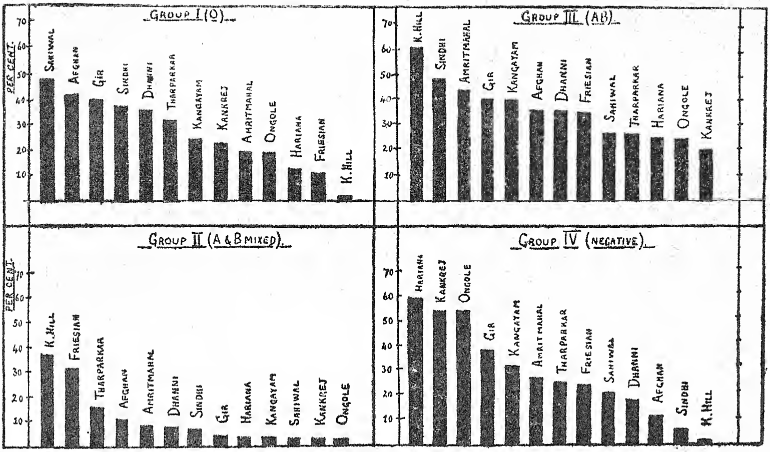

FIG. 2. Blood-group distribution of 13 breeds of cattle in India

Discussion

The value of blood-grouping for determining questions of heredity has been established by various workers. It has been found that in any race in the absence of crossing the proportion of groups remains constant from one generation to another. By crossing, a definite change in the normal proportion of groups is brought about. Before discussing the results obtained in this study, it is worth while repeating Snyder's [1930] postulates on the subject, which are as follows:

1. Any people whose blood-group distribution is studied shows blood-group frequencies similar to those of other people known to be related to it.

2. If any people shows blood-group frequencies different from those based on the frequencies of peoples known to be related to it, the conclusion may be drawn that the former has undergone racial crossings which the latter have not undergone or *vice versa*.

3. If any people shows blood-group frequency similar to a group of people not known to be related to it the conclusion may be drawn that the former traces back to the latter somewhere in its ancestry or the former has undergone crossing with the latter group or some similar people.

4. If any people lacks one or both of the blood-group mutations as evidenced by an extremely low value of p or q it may be assumed that this people became isolated from the rest of mankind before the respective mutations took place or before they spread very far.

Limitations of time and space preclude the possibility at this stage of our work of drawing a thoroughly comprehensive picture of the relationship of the various Indian cattle. It is hoped that data provided here will form the basis for further work and indicate lines of research. Nevertheless, certain interesting points emerge, which may be discussed briefly.

Kumauni hill breed. The blood-group frequencies of this breed reveal striking differences from those of the other Indian breeds so far tested. The absence of relationship to any other Indian breed, particularly in the non-appearance of group **O**, suggests a distinct origin. The Mongolian wave of immigration (Census of India, 1931 I(1)) from the Chino-Tibetan border into the Himalaya, Assam and Burma may have been instrumental in introducing these cattle into these ranges,

These small cattle are confined to the Himalayan sub-ranges and these are connected with the Tibetan ranges. It would be interesting to study the blood-groupings of Tibetan and other Mongolian breeds of cattle, or at least other Himalayan hill cattle in India, to see whether geographical proximity has provided the means of importation, adaptation and spread of these cattle in the Himalayan ranges. Their blood-group similarity with Friesians is suggestive, but no solid hypothesis is possible because few of the latter could be tested. It is intriguing however to find that both Friesian and Kumauni cattle are decidedly more susceptible to rinderpest than other breeds.

Sahiwal, Afghan, Gir and Red Sindhi. These breeds reveal a striking preponderance of group **O**, and an equally low frequency of groups **A** and **B** (mixed). This clearly establishes a close inter-relationship among these breeds and supports Olver's hypothesis, that the Sahiwal and Sindhi breeds are derived from contact with Afghan and Gir cattle. It is further established that their origin is distinct from the white-grey cattle of India.

Kankrej, Ongole and Hariana. In all these breeds there is a very low frequency of group **O**, whereas **AB** frequencies are fairly high and there is great similarity as regards group IV (negative). The Hariana cattle, tested at Hissar, it is understood, have a small admixture of Kankrej blood. In spite of this, there is a greater affinity between the Hariana and Ongole as regards group **O** and **AB**. Geographically, these two breeds are widely separated, the Ongole being confined to a small tract near the eastern coast of south India. These animals have no morphological similarity with any of the neighbouring breeds. Hariana cattle are situated well to the north, but show marked resemblance to the Ongole breed. The blood-group findings again provide support to the views of Olver and of Ware, as to the common origin of these breeds.

The Dhanni breed. This breed shows a fairly high frequency **O**. This factor and the location of these cattle, on the route from Afghanistan to India, suggest the incorporation of blood from Afghan or some allied breed during their evolution.

The Tharparker breed. This has a fairly high frequency of group **O** and **A** and **B** (mixed), and this suggests that the breed is a cross between the grey and Gir types of cattle. Observations on a large number of cattle of this breed revealed much variation in cranial formation. On morphological and serological grounds, these cattle cannot be granted the rank of a regular breed. Further investigations, however, may throw more light on the matter.

Amritmahal. This famous breed falls into a group comprising the Kangayam, Ongole and Kankrej breeds. The blood-group relationships to these three breeds are too close to be ignored, and this raises the interesting possibility that the breed may not after all have been imported in a separate migration wave, but may be the outcome of the magnificient efforts of the rulers of Mysore to evolve an army transport beast by the crossing of local south India cattle (Kangayam breed) with grey-white cattle (Ongole-Kankrej). Otherwise, there must be some common ancestry in their original home.

Summary

1. The blood-group distribution of 11 Indian and two foreign breeds, comprising 1,199 head of cattle, has been studied. With the exception of Friesians, all the cattle tested were of Zebu type.

2. The final blood-grouping by percentages is: group **O**, 26·8; group **A** and **B** (mixed), 8·4; group **AB**, 36 and group IV (negative), 28·8. The blood-group frequencies for each breed behave as independent units and indicate possible origin and relationship with other breeds.

3. Gir, Red Sindhi, Sahiwal and Afghan cattle show a similarity in blood-grouping, especially in respect of the high frequency of group **O**, so that these four breeds may be regarded as related.

Hariana, Ongole, Kankrej and Amritmahal were also very similar to one another and so may be from the same stock or closely related.

Dhanni, Tharparker and Kangayam breeds represent intermediate types.

Kumauni hill cattle appear to be a separate entity in India. Their blood-group similarity to that of Friesian cattle and the high susceptibility of both these breeds to rinderpest has been pointed out.

4. A blood-grouping classification for Indian cattle has been provisionally fixed, and a differentiation of **A** and **B** groups by absorption tests has been made. The ratio of groups **A** and **B** in Kumauni hill cattle was 2 : 1.

5. Attention has been called to the geographical distribution and morphological characters of Indian breeds.

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STUDIES ON THE COMPOSITION OF THE BLOOD OF FARM ANIMALS IN INDIA

II. SEASONAL VARIATIONS IN THE BLOOD OF DAIRY CATTLE

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IN recent years a good deal of attention has been given to the composition of blood in relation to health and disease in animals. It has been shown that malnutrition and disease often cause changes in some blood constituents. A systematic investigation was, therefore, started to establish normal values for the blood constituents of farm animals, especially cattle. In Part I of this series of papers [Mullick and Pal, 1943], an effort was made to establish normal values for blood and serum constituents of cattle belonging to different age groups, sexes, and under different physiological conditions, namely lactation, accompanied or not by pregnancy. This paper deals with the effect of seasonal variations on the morphological and chemical constituents of the blood and serum of cattle.

From the relevant literature it is observed that there is a close relationship between various body processes and changes of season. Ritzman and Benedict [1938] found that some seasonal factors exerted a dominant influence in their standard metabolism experiments. Brooks [1931] and Becker and Dix Arnold [1935] reported a close inverse relation between environmental temperature and the percentage of butter-fat in cow's milk. Similar observations were made, in India, by Sen and Rai Sircar [1944]. Seidell and Fenger

[1913] found that the average iodine-content of the healthy thyroid of cattle from June to November was about three times that from December to May.

In India, especially in the north, there is a vast difference between the winter minimum and summer maximum temperatures. At Izatnagar, the summer maximum reaches 115°F. and the winter minimum may be 42°F.

Experimental

The experimental animals were normal adult cows of the Hariana breed belonging to the Izatnagar Dairy Farm. They were selected because their blood constituents had remained constant under various normal physiological conditions [Mullick and Pal, 1943]. Feeding and management were under supervision. All had been immunized against rinderpest and were tuberculin-negative; they remained in sound health throughout the observations (November 1941 to October 1942). Since proposed fortnightly examinations were not possible, analyses were made monthly.

Blood samples were taken in the morning before feeding and at practically the same period every month. Oxalated blood was analysed for red cells, white cells, hæmoglobin, cell volume, iron, inorganic phosphorus, sugar and cholesterol. Calcium, magnesium, protein and non-protein-nitrogens were estimated in the serum. Samples for inorganic phosphorus and sugar were deproteinized at the spot of bleeding.

Methods

Cell-volume, cholesterol, protein and non-protein nitrogens were determined by the methods of Napier and Das Gupta [1941], Banerjee [1937] and Kjeldahl [1883], respectively. The rest of the constituents were analysed according to the methods described in Part I [Mullick and Pal, 1943].

Results and discussion

The average readings of all the animals for each month are given in Table I, of individual animals for 12 months and the results of statistical analysis in Table II.

TABLE I

Seasonal variation in the composition of blood and serum of cows

(Average of 12 animals)

—	1941		1942									
	Nov.	Dec.	Jan.	Feb.	Mar.	Apr.	May	June	July	Aug.	Sep.	Oct.
Red cells, millions per c.mm. blood	8·4	8·1	8·3	8·1	8·4	8·2	8·2	8·1	8·2	8·1	8·0	8·3
White cells, thousands per c.mm. blood	9·4	9·0	8·8	9·2	9·2	9·1	9·1	9·3	9·5	9·2	9·0	9·1
Hb., gm. per 100 c.c.	11·1	10·8	11·1	10·9	10·5	10·0	9·9	9·8	10·1	10·4	10·8	11·5
C. V. per cent	40·7	41·1	39·8	39·0	38·3	36·9	36·1	35·8	37·5	38·3	39·9	41·1
M. C. V. cu. μ	48·9	50·9	47·9	48·2	45·9	45·1	43·9	44·2	45·6	45·8	49·9	50·1
M. C. H. γγ	13·4	13·4	13·3	13·3	12·6	12·3	12·0	12·2	12·3	12·5	13·4	13·9
M. C. H. C. per cent	27·5	26·4	27·9	27·9	27·3	27·2	27·5	27·5	26·9	[illegible]	27·1	28·0
Iron mg. per cent	40·6	39·8	40·1	39·7	38·7	39·1	38·6	38·9	39·8	40·1	39·1	42·9
Inorganic phosphorus mg. per cent	4·35	4·61	4·72	4·53	4·51	4·81	4·37	4·46	4·43	4·31	4·50	4·53
Sugar, mg. per cent	82·3	80·8	81·9	80·0	81·1	80·5	80·0	80·6	80·6	80·2	78·7	79·3
Cholesterol, mg. per cent	146	144	139	143	144	144	137	135	138	141	139	136
Calcium, mg. per cent	10·5	10·2	10·1	10·6	10·5	10·7	11·5	11·5	11·3	11·1	10·8	11·0
Magnesium, mg. per cent	2·92	2·57	2·71	2·80	2·57	2·61	2·69	2·71	2·73	2·70	2·61	2·63
Non-protein nitrogen mg. per cent	35·9	35·4	36·0	36·1	36·4	35·6	36·5	36·5	37·7	34·4	33·4	36·4
Protein gm. per cent	7·3	7·3	7·3	7·2	7·4	7·3	7·1	7·1	7·4	7·6	7·2	7·3

TABLE II

Seasonal variation in the composition of blood and serum of cows and statistical analysis

(Average of 12 months)

Animal No.	22	23	27	42	57	61	94	111	123	126	145	178	Mean	S. D.	C. of var.
Red cells . . .	8·4	7·8	8·2	8·6	8·0	8·2	8·0	7·9	8·6	8·4	8·4	8·2	8·2	±0·3855	4·7
White cells . . .	9·2	9·2	9·2	9·7	9·3	9·2	9·3	8·7	9·0	8·7	0·0	9·5	9·2	±0·5639	6·1
Hb.	10·3	10·2	10·5	10·9	10·4	10·3	10·2	10·7	11·4	10·3	11·1	10·6	10·6	±0·4942	4·7
C. V.	38·5	7·5	37·9	39·5	38·3	38·5	37·0	38·3	40·3	38·5	40·2	40·0	38·7	±1·4709	3·8
M. C. V. . .	46·2	48·3	46·5	45·9	47·7	47·1	46·0	48·6	46·8	46·1	48·1	49·1	47·2	±2·9120	6·2
M. C. H. . . .	12·4	13·2	12·8	12·6	12·9	12·6	12·7	13·4	13·2	12·3	13·3	13·0	12·9	±0·7578	5·9
M. C. H. C. . . .	26·7	27·4	27·8	27·5	27·1	27·0	27·6	27·8	28·5	26·8	27·6	27·8	27·4	±1·6707	6·1
Iron	39·4	37·9	38·4	41·1	40·4	39·8	39·5	39·7	40·8	40·6	40·4	40·1	39·8	±1·5761	4·0
Inorganic phosphorus .	4·30	4·38	4·50	4·68	4·47	4·37	4·46	4·67	4·60	4·46	4·14	4·92	4·51	±0·4723	10·5
Sugar	79·1	80·6	80·5	80·6	81·1	81·8	81·1	78·7	80·6	81·2	79·8	81·2	80·5	±3·1221	3·9
Cholesterol . . .	151	150	145	136	143	136	138	142	136	136	134	141	141·0	±11·6685	8·3
Calcium	11·0	10·9	11·3	10·8	10·9	10·9	10·8	10·1	10·1	10·18	11·1	10·6	10·8	±0·4579	4·2
Magnesium . . .	2·78	2·75	2·85	2·65	2·74	2·60	2·65	2·77	2·51	2·64	2·65	2·72	2·69	±0·1839	6·8
Non-protein nitrogen .	31·5	36·4	36·5	34·1	35·1	36·3	36·5	36·3	34·9	36·6	36·6	36·2	35·9	±2·3677	6·6
Protein	7·4	7·1	7·5	7·1	7·6	7·6	7·6	7·3	7·3	7·2	7·3	7·2	7·3	±0·3928	5·4

Red corpuscles and white corpuscles. From the tables, it will be seen that the average values remained practically constant throughout the year. However, highly significant differences were found among individual animals.

Hæmoglobin. Highly significant differences existed in the monthly averages as well as in the means of individual animals. Monthly averages declined gradually from February to June, and from July they started to increase. It appears that the concentration of hæmoglobin is inversely proportional to the change of atmospheric temperature. In South Africa, Hammersma [1937] studied the seasonal variation of hæmoglobin in animals and found no significant variation in monthly averages. Manresa and Orig [1941] in the Philippines observed that the hæmoglobin concentration in experimental animals was higher in winter than in summer. In the United States Bazett Sunderman, Doupe and Scott [1940], working on human subjects, found that a slight variation in the temperature of the experimental chamber resulted in variation in the concentration of blood constituents, the trend of changes being similar to that observed by us.

Cell-volume. The variation of cell-volume as a result of change in air temperature resembled that of hæmoglobin. Statistical analysis showed that there was highly significant difference among the monthly averages as well as among the means of individual animals. This value was inversely proportional to the change of temperature. The variation in the composition of body-fluid with seasonal change helps heat-regulation in the animal. In summer, the secretions of thyroxine and adrenaline temporarily inhibit dilation of the cutaneous capillaries and the increase in plasma volume helps in giving out the body heat formed in excess [Kuno, 1935].

Mean corpuscular volume. The average monthly values showed highly significant variations, but the means of individual animals did not differ significantly. Monthly variation was similar to that of cell-volume.

Mean corpuscular hæmoglobin. Highly significant differences existed among the monthly averages as well as in individual animals. The values were higher in winter than summer.

Mean corpuscular hæmoglobin concentration. The average values showed no significant variation in the various months or in the individual animals. The change in both hæmoglobin and cell-volume was shown to be proportional.

Iron. There were highly significant differences among the monthly averages and in individual animals. No seasonal variation was noted by Hammersma [1937] in South African animals. Considering the close relationship between hæmoglobin and iron, it might have been expected that variation in iron would be similar to that of hæmoglobin.

Inorganic phosphorus. There was no significant difference among the monthly averages but mean values of the individual animal differed significantly.

Sugar. The mean values showed no significant differences in the monthly observations or in individual animals.

Cholesterol. Neither the monthly averages nor the means of the individual animals varied significantly. Ghosh [1933] came to a similar conclusion from a statistical study of the seasonal variation in human blood.

Calcium. There were highly significant differences in both the means of monthly observations and in the averages of individual animals. Monthly values gradually increased with rise of temperature in summer and gradually decreased with the advance of winter. Hammersma [1937] noted similar changes in cattle, but the difference was not statistically significant. Craige and Godd [1941] found the calcium content of horse's serum to be at its maximum in summer.

Magnesium. Highly significant differences existed among the monthly averages as well as in individual animals. Duncun, Lightfoot and Huffman [1940] noted also that this constituent differed significantly from month to month, winter values being greater than summer ones.

Protein and non-protein nitrogen. Neither the average monthly values nor the means of individual animals were found to be significantly different. Hammersma's [1937] findings were similar to ours.

Summary

Whole-blood and serum of a group of normal cows were analysed for morphological and chemical constituents over a period of 12 months. Oxalated blood was examined for red cells, white cells, hæmoglobin, cell-volume, iron, inorganic phosphorus, sugar and cholesterol. Calcium, magnesium, protein and non-protein nitrogen were determined in serum. Different corpuscular values were calculated from red cell counts, hæmoglobin and cell volume. In the morphological series, hæmoglobin and cell-volume exhibited significant seasonal variations, whereas in the chemical series monthly averages for iron, calcium and magnesium showed significant differences. In the former series, the average values were highest in winter, whereas in the latter series they were highest in summer. The remaining constituents, both in the morphological and chemical series, showed no significant differences in their monthly averages. Practically all the constituents differed significantly in individual animals.

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THE COMPARATIVE VALUE OF SOME CONCENTRATES IN THE FEED OF GROWING CATTLE

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(With one text-figure)

Concentrates can be broadly classified into three groups according to the composition. Linseed cake, decorticated and undecorticated groundnut cakes, *til* (*Sesamum indicum*) cake, rape cake, cottonseed cake and *guar* (*Cyamopsis psoralioides*) are the common concentrates of India, having a protein content of over 30 per cent. Coconut cake, cotton seed, pulses (legume seeds) and pulse by-products are moderately rich in protein and have nutritive ratios similar to the standard required for production. Cereal grains make the third group of concentrates which are comparatively rich in carbohydrates but low in protein content.

This paper deals with the feeding value, for the production of growth, of five oil cakes, viz. linseed cake, decorticated and undecorticated groundnut cakes, rape cake and *til* cake and four legume seeds or their by-products, viz. *guar*, gram (*Cicer arietinum*), *arhar* (*Cajanus indicus*) *chuni* and *mung* (*Phaseolus mungo*) *chuni*. The *chuni* is the by-product from the manufacture of split pulse (*dal*) for human consumption. It contains chiefly the hulls, along with the germs and broken particles of the seed. In all the tests of this series, linseed cake was used as the control, because it is one of the most popular protein supplements for cattle and produces good results even when used as the only concentrate.

Feeding trial with linseed cake, undecorticated groundnut cake, gram and arhar *chuni* (1940)

In August 1940, tests to find out the comparative feeding values of linseed cake, undecorticated groundnut cake, gram and *arhar chuni* for growth were started with 20 Hissar heifers. The basal ration, which consisted of wheat straw *ad lib.*, 11 lb. *jowar* (*Sorghum vulgare*) silage, 1·55 lb. wheat bran and linseed cake for protein supplement, was fed to all the animals for a period of eight weeks and thereafter they were distributed into four comparable groups according to age, liveweight and rate of growth during the basal period. For the experimental feeding, group I was allowed to continue the basal ration and groups II, III and IV received groundnut cake, gram and *arhar chuni* respectively in place of linseed cake, the quantity being regulated to maintain the same level of protein for different groups. Records of daily liveweight and food consumption for each animal were maintained during the test which lasted for a period of 21 weeks. The distribution of animals for the different treatments and their liveweight increase during the experimental period have been recorded in Table I.

Rate of growth with the different rations. The rate of growth by the different treatments has been statistically analysed and the summary of the results is shown in Table II. Undecorticated groundnut cake produced the lowest average growth rate. Linseed cake came next and, though the average growth rate is very significantly higher than that with groundnut cake, it is lower than that with gram and *arhar chuni*. The difference between gram and *arhar chuni* is not significant. The fall in the growth rate from gram to linseed cake is 19·48 per cent, from gram to groundnut cake is 53·60 per cent, and from linseed cake to groundnut cake 34·12 per cent, the critical difference at 5 per cent level being 12·97 per cent. Similarly, between *arhar chuni* and linseed cake and between *arhar chuni* and undecorticated groundnut cake the differences in the average growth rates are 23·35 and 57·47 per cent respectively, the critical difference being 14·50 per cent. The variations due to treatments in the case of average rate of change of growth rate is also significant.

Feeding trial with linseed cake, decorticated groundnut cake, rape cake and mung *chuni* (1941)

Sixteen Hissar heifers were selected for this test in September, 1941. The basal ration was the same as in the previous test, except that rape cake was fed instead of linseed cake during this period as it was found that animals do not relish

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TABLE I

Distribution of the animals into different groups and their liveweight (lb.)

	Linseed cake					Groundnut cake (undecorticated)				
	1	2	3	4	5	6	7	8	9	10
Age on 28 August 1940 (year, months and days)	1-8-24	1-8-18	1-7-9	1-7-13	1-8-13	1-8-27	1-7-29	1-9-5	1-7-3	1-6-20
Average age of the group .			1 year 7 months 3 days					1 year 7 months 1 day		
Average liveweight at the start of the experiment on 28 August 1940	519	452	450	422	457	483	486	443	472	425
Average liveweight of the group			460					462		
Average liveweight at the close of the experiment on 21 January 1941	627	595	623	552	604	559	595	539	587	535
Total increase . . .	108	143	173	130	147	76	109	96	115	110
Average increase of the group			140					101		
	Gram					*Arhar chuni*				
	11	12	13	14	15	16	17	18	19	20
Age on 28 August 1940 (year, months and days)	1-8-5	1-7-3	1-9-9	1-7-19	1-8-24	1-8-0	1-8-7	1-8-12	1-8-3	1-7-14
Average age of the group .			1 year 7 months 6 days					1 year 7 months 1 day		
Average liveweight at the start of the experiment on 28 August 1940	480	473	451	460	427	513	492	413	424	472
Average liveweight of group			460					475		
Average liveweight at the close of the experiment on 21 January 1941	684	672	634	596	575	662	682		598	636
Total increase . . .	195	199	183	136	148	149	190		174	164
Average increase of group .			172					170		

TABLE II

Summary of results (rate of growth by different treatments)

Treatments	Linseed cake	Groundnut cake (undecorticated)	Gram	*Arhar chuni*	Mean
Average increase per week (lb.)	7·11	4·66	8·51	8·79	7·19
Percentage of the mean	98·94	64·82	118·42	122·29	100·00
Critical difference at 5 per cent	0·93	0·93	0·93	1·04	. .
Per cent critical difference of mean . . .	12·97	12·97	12·97	14·50	. .
Rate of change of growth rate	—0·0806	—0·0109	—0·1416	—0·1916	—0·1017
Percentage of mean	79·21	10·74	139·23	188·43	100·00
Critical difference at 5 per cent	0·0757	0·0757	0·0757	0·0846	. .
Per cent critical difference of mean . . .	74·39	74·39	7·349	83·18	. .

a change from linseed cake to rape cake. The basal feeding lasted for three weeks, after which the animals were distributed into four comparable groups. One group was allowed to continue the rape cake ration, and for the other three groups rape cake was replaced by either linseed cake, groundnut cake or *mung chuni*. The procedure was the same as in the previous test. The distribution of the animals for the different treatments and the gain in their liveweights during the 18 weeks of experiment are shown in Table III.

Rate of growth. The results of the statistical analysis have been summarized in Table IV. Decorticated groundnut cake has produced significantly the lowest average growth rate. Rape cake and linseed cake come next in order and the difference between them is not significant, but both produced significantly lower growth rate than *mung chuni*, the average growth rates per week with these feeds being 4·96, 6·30, 6·84 and 8·72 lb. respectively. The fall in the growth rate from *mung chuni* to rape cake is 36·03 per cent, from *mung chuni* to groundnut cake is 56·04 per cent, and from rape cake to groundnut cake 20·01 per cent, the critical difference at 5 per cent level being 19·64 per cent. Similarly, from *mung chuni* to linseed cake and from linseed cake to groundnut cake, the fall in the average growth rate is 27·90 and 28·14 per cent respectively. The variation due to treatments in the average rate of change of growth rate is also significant.

Table III

Distribution of animals into groups and their average liveweight (lb.)

	Linseed cake				Groundnut cake (decorticated)			
	1	2	3	4	5	6	7	8
Age on 11 October 1941 (year, months and days).	1-7-28	1-7-4	1-6-15	1-6-11	1-7-18	1-7-12	1-6-21	1-6-15
Average age of group		1 year 7 months				1 year 7 months 1 day		
Average liveweight at the start of the experiment on 11 October 1941	407	432	363	416	414	376	403	430
Average liveweight of the group		404				406		
Average liveweight at the close of the experiment on 14 February 1942	530	582	497	533	509	471	506	513
Total increase	123	150	134	117	95	95	103	83
Average increase of the group		131				94		
	Rape cake				*Mung chuni*			
	9	10	11	12	13	14	15	16
Age on 11 October 1941 (year, months and days)	1-7-18	1-7-3	1-6-27	1-6-2	1-7-24	1-7-7	1-6-19	1-6-14
Average age of group		1 year 6 months 27 days				1 year 7 months 1 day		
Average liveweight at the start of the experiment on 11 October 1941	437	401	396	376	380	440	427	395
Average liveweight of the group		402				404		
Average liveweight at the close of the experiment on 14 February 1942	541	498	533	496	549	587	583	552
Total increase	104	97	137	120	169	173	156	157
Average increase of the group		115				164		

TABLE IV

Summary of results (rate of growth by different treatments)

Treatments	Linseed cake	Groundnut cake (de-corticated)	Rape cake	*Mung chuni*	Mean	Critical difference at 5 per cent
Average increase per week (lb.) . .	6·84	4·96	6·30	8·72	6·705	1·317
Percentage of the mean . .	102·10	73·96	93·97	130·00	100·00	19·64
Rate of change of growth rate . .	—0·1591	—0·0438	—0·0913	—0·2479	—0·1355	—0·1339
Per cent rate of change of growth rate .	117·40	32·32	67·39	183·00	100·00	98·45

FEEDING TEST WITH LINSEED CAKE, *til* CAKE AND *guar* (1942)

The experiment to study the comparative feeding values of linseed cake, *guar* and *til* cake was started in April, 1942 with 15 Hissar heifers. The constituents of the basal ration were the same as those during the first test of the series. The experiment was conducted for 16 weeks after a preliminary feeding for four weeks. There were five replications for each treatment. The distribution of the animals in three groups and their liveweight increase during the experiment have been recorded in Table V.

TABLE V

Distribution of animals into different groups and their liveweight (lb.)

	Linseed cake					*Til* cake					*Guar*				
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Age on 21 April 1942 (year, months and days)	1-8-12	1-5-9	1-4-21	1-4-8	1-3-23	1-6-2	1-5-27	1-5-12	1-4-18	1-4-2	1-7-17	1-5-13	1-5-4	1-4-14	1-3-22
Average age of the group	1 year 5 months 8 days					1 year 5 months 6 days					1 year 5 months 8 days				
Average liveweight at the start of the experiment on 21 April 1942	429	346	395	316	354	383	369	426	317	341	427	388	372	300	360
Average liveweight of group.			368					367					369		
Average liveweight at the close of the experiment on 11 August 1942	535	422	503	449	465	457	487	508	418	394	556	509	503	452	496
Total increase . .	106	76	108	133	111	74	118	82	101	53	129	121	131	152	136
Average increase of the group			107					86					134		

Rate of growth. The summary of results of statistical analysis for the rate of growth is given in Table VI. It is observed that the rate of growth with *til* cake is significantly lower than that with linseed cake or *guar*. The fall in the rate of growth from linseed cake to *til* cake is 25·49 per cent and from *guar* to *til* cake 43·18 per cent. *Guar* has produced the highest rate of growth and the fall in the rate of growth from *guar* to linseed cake is 17·69 per cent, but it is not significant being less than the critical difference of 24·59 per cent.

TABLE VI

Summary of results for rate of growth

Feeds	Linseed cake	*Til* cake	*Guar*	Mean	Critical difference at 5 per cent
Average increase per week (lb.)	6·85	5·15	8·03	6·67	1·64
Percentage of the mean.	102·70	77·21	120·39	100·00	24·59

COMPARATIVE RATE OF GROWTH WITH THE DIFFERENT CONCENTRATES

Striking differences in the average growth rates due to the different treatments can be seen when the experimental data are plotted on the graph (Fig. 1). The curves for groundnut cakes, both decorticated and undecorticated, rape cake and *til* cake are less steeply inclined than those with linseed cake. Better rates of growth are to be found with *arhar chuni*, gram, *mung chuni* and *guar*. These findings are substantially confirmed by the statistical analysis. The data obtained during the three years of experiment have been analysed to study the comparative feeding value of the concentrates. The summary of the results is shown in Table VII.

The differences in the rates of growth obtained with linseed cake during the three experiments are not significant yet they are not the same which may be due to seasonal variations or other factors during the three different years. It is observed from Table VII that the average rates of growth with undecorticated and decorticated groundnut cakes are not significantly different from that with *til* cake but are significantly lower than that with the remaining concentrates. The growth rate with *til* cake is not significantly different from that with rape cake, but is lower than those with linseed cake, *guar*, gram, *arhar chuni* and *mung chuni*. The growth with rape cake is not significantly lower than that with linseed cake, but is lower than the figures obtained for *guar*, gram, *arhar chuni* and *mung chuni*. The rate of growth with linseed cakes is not significantly different from that with *guar*, but is lower than those with gram, *arhar chuni* and *mung chuni*. The differences obtained among *guar*, gram, *arhar chuni* and *mung chuni* are not significant. The results can be symbolically represented as follows:

Groundnut cake (undecorticated)	4·58
Groundnut cake (decorticated)	4·96
Til cake	5·15
Rape cake	6·30
Linseed cake (1942)	6·83
Linseed cake (1941)	6·87
Linseed cake (1940)	7·13
Guar	8·03
Gram	8·77
Arhar chuni	8·79
Mung chuni	8·83

The variations observed in the growth rate may be due to higher consumption, better digestibility of the total ration on account of the presence of certain concentrates or superior value of the nutrients of some concentrates.

Consumption of total ration as influenced by the different concentrates. The average consumption per animal per day in all the treatments and the intake per 100 lb. of liveweight have been tabulated in Table VIII. The average composition of the feeds during the experiment is given in Table IX.

TABLE VII

Summary of results of combined analysis

	1940				1941				1942			Mean	Critical difference 5 per cent.*		
	Linseed cake	Groundnut cake undecorticated	Gram	*Arhar chuni*	Linseed cake	Groundnut cake decorticated	Rape cake	*Mung chuni*	Linseed cake	*Til* cake	*Guar*		Treatments with 4 animals	Treatments with 5 animals	Treatments one with 4 and the other with 5 animals
Average increase per week (lb.).	7·13	4·58	8·77	8·79	6·87	4·96	6·30	8·83	6·83	5·15	8·03	6·91	1·50	1·35	1·43
Percentage of mean	103·18	66·28	126·92	127·21	99·42	71·78	91·17	127·79	98·84	74·53	116·21	100·00	21·71	19·54	20·69

* Four animals under each treatment in 1940 and 1941 and 5 in 1942 were considered for the combined analysis.

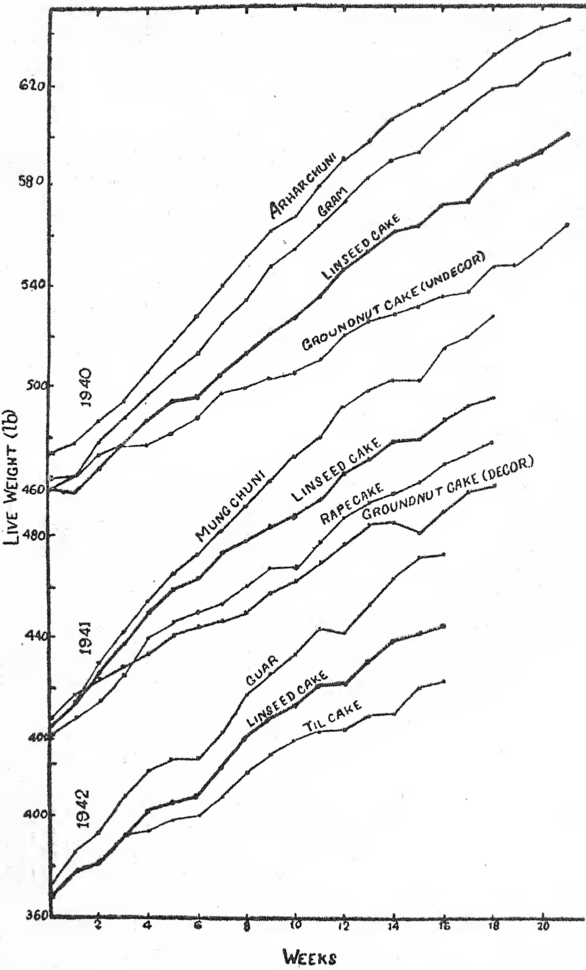

FIG. 1. Rate of growth with different concentrates

Table VIII

Intake of total dry matter per day with the different concentrates (lb.)

Treatments	Wheat straw	Silage	Wheat bran	Concentrates	Total	Average live-weight	Intake per 100 lb. live-weight
1940							
Linseed cake	2·92	4·08	1·40	2·95	11·35	530	2·14
Groundnut cake (undecorticated)	2·70	4·07	1·40	2·98	11·15	512	2·18
Gram	2·67	4·06	1·40	4·39	12·52	546	2·29
Arhar chuni	2·49	4·07	1·38	5·34	13·28	560	2·37
1941							
Linseed cake	2·45	4·28	1·41	2·82	10·96	470	2·33
Groundnut cake (decorticated)	2·42	4·27	1·41	1·48	9·58	452	2·12
Rape cake	2·39	4·25	1·41	2·45	10·50	460	2·28
Mung chuni	1·64	4·26	1·41	5·31	12·62	486	2·60
1942							
Linseed cake	1·73	4·41	1·41	2·21	9·76	427	2·29
Til cake	1·82	4·39	1·37	1·61	9·19	415	2·21
Guar	1·67	4·37	1·39	3·05	10·48	444	2·36

Table IX

Average percentage composition of feeds on dry basis

Feeds	Organic matter	Protein	Ether extract	Fibre	N-free extract	Total carbohydrates
1940						
Wheat straw	91·2	2·01	0·675	39·9	48·7	88·5
Jowar silage	88·3	3·27	0·618	36·1	48·2	84·3
Wheat bran	93·6	15·06	3·163	11·2	64·2	75·4
Linseed cake	92·3	31·05	7·033	8·4	45·1	54·5
Groundnut cake (undecorticated)	87·0	31·57	9·279	20·9	25·3	46·2
Gram	96·0	21·90	4·015	9·5	60·6	70·1
Arhar chuni	91·6	19·36	1·542	19·0	51·7	70·7
1941						
Wheat straw	90·4	2·37	0·865	39·3	47·9	87·2
Jowar silage	88·4	5·30	1·702	31·6	49·8	81·4
Wheat bran	94·6	12·50	3·397	13·0	65·7	78·7
Linseed cake	90·0	33·03	3·082	9·0	44·9	53·9
Groundnut cake (decorticated)	92·5	52·90	7·647	6·7	25·2	31·9
Rape cake	90·6	37·74	8·121	8·1	36·7	44·8
Mung chuni	89·1	22·24	1·253	15·0	50·6	65·6
1942						
Wheat straw	92·1	3·04	0·510	42·8	45·8	88·6
Jowar silage	88·4	5·11	1·426	32·5	49·5	82·0
Wheat bran	92·0	14·53	3·459	11·8	62·2	74·0
Linseed cake	93·1	36·56	3·910	9·4	43 3	52·7
Til cake	87·0	46·78	7·364	5·0	27·9	32·9
Guar	95·4	30·56	2·940	10·0	51·9	61·9

Smaller quantities of concentrates richer in protein were fed to maintain the same level of protein for all the treatments, and it is noted that the consumption of total dry matter was also lower in these groups. Concentrates and coarse fodders were fed separately. Where the quantity of concentrate was comparatively higher, as with *arhar chuni* and *mung chuni*, the intake of wheat straw was slightly lower, but in most of the treatments its intake, though fed *ad lib*, did not appreciably increase with the lower amount of concentrates, which indicates that the animals were usually satiated with the average total dry matter consumed in the oil cake treatments. Thus it appears that on an average 2·22 lb. total dry matter per 100 lb. liveweight is necessary for Hissar heifers between one and two years of age and weighing between 400 and 500 lb., but the rate of consumption may be higher if the ration consists mostly of palatable feeds. In the *mung chuni* treatment, the average consumption per 100 lb. liveweight was as high as 2·60 lb. The higher intake of dry matter must have influenced, to some extent, the better growth rate with *guar*, gram, *arhar chuni* and *mung chuni*.

Effect of the concentrate on the digestibility of the total ration. Digestibility trials were conducted with three animals under each treatment during 1940 and 1941 and with two animals in 1942. The average digestibility coefficients of the nutrients of the total ration for each treatment have been recorded in Table X.

TABLE X

Digestibility of total ration with the different concentrate (per cent)

Treatments	Organic matter	Protein	Ether extract	Fibre	N-free extract	Total carbohydrates
1940						
Linseed cake	59·2	62·8	77·3	56·2	55·7	55·7
Groundnut cake (undecorticated)	55·3	63·2	79·6	51·2	54·7	54·5
Gram	65·0	59·7	72·6	61·8	66·6	65·0
Arhar chuni	62·7	50·9	65·1	71·0	60·9	65·0
1941						
Linseed cake	61·2	61·9	61·7	59·7	59·9	59·9
Groundnut cake (decorticated)	59·9	63·1	65·3	59·4	56·3	57·4
Rape cake	60·5	63·1	73·5	58·7	58·1	58·3
Mung chuni	62·1	56·5	47·4	58·5	63·9	62·1
1942						
Linseed cake	60·1	68·2	58·6	56·0	60·6	59·0
Til cake	58·2	68·3	71·5	54·2	56·8	55·9
Guar	64·0	68·3	63·7	57·9	66·0	63·2

When gram, *guar* or pulse by-products are present in the ration both organic matter and total carbohydrates are better digested, whereas the digestibility of protein is superior when concentrates richer in protein are fed.

This indicates that the concentration of a nutrient in the total ration plays an important role in its digestibility. The level of protein was the same in all the treatments, but due to a lower intake of total dry matter the concentration of protein was higher in the treatments with feeds richer in protein, resulting in its better digestibility. The same tendency is noticed with the digestibility of ether extract. Similar observations were made by the author [1943] on a previous occasion. The lower digestibility of protein of the total ration with gram, *arhar chuni* and *mung chuni* might also have been influenced by the presence of more easily digestible carbohydrates.

Gain in liveweight in relation to the intake of digestible nutrients. The increases in liveweight per unit of digestible protein and total digestible nutrients have been calculated (Table XI) to compare the feeding value of the nutrients from the different concentrates. The pulses and pulse by-products have always given higher increase in

liveweight per unit of digestible protein. This greater increase cannot be attributed only to the quality of protein, as more carbohydrates also had to be ingested as a result of maintaining the same level of protein in these treatments. Balancing of both protein and carbohydrates, which is necessary for such experiments, is only possible by supplementing purified diets, but this is not practicable specially with dairy cattle, in long period feeding trials. In the experiment during 1940, the actual amount of protein digested was lower than the calculated quantity given to the animals of gram and *arhar chuni* groups, and hence, the level of protein supplement was lower for these two treatments. But the increase in liveweight per unit of total digestible nutrients was the same even with this lower protein supplement. *Mung chuni* and *guar* in 1941 and 1942 respectively have produced decidedly better rate of growth per unit of both digestible protein and total digestible nutrients. Thus it appears that for growing animals protein from gram, *arhar chuni*, *mung chuni* or *guar* is superior to that from the oilcakes.

TABLE XI

Gain in liveweight in relation to the intake of digestible nutrients (lb.)

Treatments	Liveweight increase	D. P. intake	T. D. N. intake	Increase per lb. of D. P.	Increase per lb. of T. D. N.	Average liveweight
1940						
Linseed cake	140	121·9	911·8	1·15	0·15	530
Groundnut cake (undecorticated)	101	124·4	868·3	0·82	0·12	513
Gram	172	119·1	1132·8	1·44	0·15	546
Arhar chuni	170	106·6	1132·8	1·60	0·15	560
1941						
Linseed cake	131	108·6	770·9	1·21	0·17	470
Groundnut cake (decorticated)	94	97·3	663·6	0·98	0·14	452
Rape cake	115	110·0	741·5	1·05	0·16	460
Mung chuni	164	115·4	886·5	1·42	0·19	486
1942						
Linseed cake	107	98·6	616·2	1·09	0·17	427
Til cake	86	94·1	559·7	0·91	0·15	415
Guar	134	107·6	707·7	1·25	0·19	444

Relative economy of the concentrates

The cost for production of 100 lb. liveweight with all the concentrates is shown in Table XII.

The tests were carried out for three consecutive years and the prices of feeds varied from year to year. Hence, to compare the cost of production with the different concentrates, the prices of all the roughages and bran have been taken as the same as in 1941 and those of the concentrates have been modified and brought to the 1941 level on the basis of variation of the price of linseed cake from year to year. The comparative cost of the total ration for the production of 100 lb. liveweight (Table XII) show that for production of the same liveweight rape cake is the cheapest amongst all the concentrates, next in order come groundnut cake (decorticated), *guar*, *mung chuni*, linseed cake, *til* cake, *arhar chuni*, groundnut cake (undecorticated) and gram.

The comparative economic value of a feed cannot be assessed only by the market prices which always vary. Therefore the quantities of different concentrates which produce the same increase in liveweight have been shown in the last column (Table XII). But for true economy the time factor should also be taken into account as with better rate of growth, the period of unproductiveness is shortened. It appears that when the rate of growth is taken into account *guar* is definitely more economical than the oilcakes, and both *arhar chuni* and *mung chuni* may also prove cheaper in the long run.

Summary

Trials were conducted to study the comparative feeding values of linseed cake, decorticated and undecorticated groundnut cakes, rape cake, *til* cake, *guar*, gram, *arhar chuni* and *mung chuni*

TABLE XII

Relative economy of the concentrates

Concentrate	Gain in liveweight	Quantity of concentrates fed	Prices per 100 lb. during experiment	Cost of total ration for 100 lb. increase in liveweight	Prices per 100 lb. on the basis of 1941 rate	Comparative cost of production of 100 lb. liveweight on the basis of 1941 prices	Equivalent quantities
	Lb.	Lb.	Rs.	Rs.	Rs.	Rs.	Lb.
1940 (21 weeks)							
Linseed cake	140	433·6	2·745	17·34	2·745	14·67	100
Groundnut cake (undecorticated)	101	438·0	1·829	19·34	1·829	16·53	135
Gram	172	645·4	3·354	20·35	3·354	17·22	120
Arhar chuni	170	784·8	2·256	17·78	2·256	15·04	137
1941 (18 weeks)							
Linseed cake	131	372·9	2·745	14·67	2·745	14·67	100
Groundnut cake (decorticated)	94	192·9	1·829	13·21	1·829	13·21	77
Rape cake	115	324·3	1·829	12·87	1·829	12·87	99
Mung chuni	164	705·3	2·073	14·01	2·073	14·01	138
1942 (16 weeks)							
Linseed cake	107	266·7	3·659	25·4	2·745	14·67	100
Til cake	86	196·4	3·049	27·3	2·288	14·75	93
Guar	134	370·5	3·659	22·9	2·745	13·92	104

with Hissar heifers. The tests were carried out in three sets during 1940, 1941 and 1942. Linseed cake was used as control in all the sets. Varying quantities of the different concentrates were fed to maintain the same level of protein in all the treatments.

In the first test, it was found that gram and *arhar chuni* produced better growth than linseed cake, while undecorticated groundnut cake proved inferior. Among the concentrates tested in the second year, *mung chuni* gave the highest rate of growth, followed in order by linseed cake, rape cake and decorticated groundnut cake. The difference in the growth rates with linseed cake and rape cake was not significant. In the third set, the growth rates were in the decreasing order of *guar*, linseed cake and *til* cake.

On comparing the data for all the concentrates, it was found that under the conditions of these experiments groundnut cakes both decorticated and undecorticated resulted in the least favourable rate of growth. *Til* cake and rape cake came next in order of increasing value, followed by linseed cake. Linseed cake was of about the same value as *guar* and was inferior only to gram, *arhar chuni* and *mung chuni* which gave the best results.

The digestibility of organic matter and total carbohydrate was higher when pulse or pulse *chuni* was present in the ration, while that of protein was superior when the concentrates richer in protein were fed. This variation appears to be due to the concentration of the nutrient in the total ration.

Protein of gram, *arhar chuni*, *mung chuni* and *guar* appears to be of superior quality as compared to that of the oilcakes for growing Hissar cattle. *Mung chuni* and *guar* produced the best rates of growth per unit of total digestible nutrients as well. For the production of the same liveweight rape cake has been found to be the cheapest. Next in order come decorticated groundnut cake, *guar*, *mung chuni*, linseed cake, *til* cake, *arhar chuni*, undecorticated groundnut cake and gram. But when the rate of growth is taken into account, which determines the period of unproductiveness the feeding of *guar* is definitely more economical than the oilcakes and both *arhar chuni* and *mung chuni* may also prove cheaper in the long run.

With normal feeds, the consumption of dry matter for Hissar heifers weighing between 400 and 500 lb. is, on an average, 2·2 lb. per 100 lb. liveweight.

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BAGOMOLASSES AS CATTLE FEED

I. DIGESTIBILITY AND NUTRITIVE VALUE OF BAGOMOLASSES

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WITH the extensive development of sugar industry in this country, the economic disposal of a large quantity of molasses in every crushing season has become a problem for urgent consideration. Of late, attempts have been made to open up several avenues of economic utilization of molasses. One of these is its possible use as a cattle-feed.

The Imperial Institute of Sugar Technology had been, for some time past, manufacturing cattle feed containing a substantial quantity of molasses. It was prepared by mixing dried bagasse screenings and protein concentrate (made of *chunni* and an oilcake) with boiled molasses. The mixture was pressed into a cake and was named as 'molasscuit'. Some feeding tests with molasscuit were carried out at several centres. Preliminary reports of these trials were somewhat contradictory as to the suitability of molasscuit as a cattle feed. The manufacture of molassed feed containing protein concentrate was eventually dropped and the Imperial Institute of Sugar Technology is now producing a new feed very similar to the foregoing in general make-up but containing no protein supplement. The new feed has been named 'bagomolasses', and is made up of one part bagasse screenings mixed with two parts of molasses.

This paper embodies the results of experiments carried out with a view (1) to record any characteristic behaviour shown by experimental animals kept on a ration containing bagomolasses, (2) to study the effect of incorporating bagomolasses on consumption, digestion and utilization of a maintenance ration by bullocks, and (3) to evaluate the digestibility coefficients and nutritive value of bagomolasses.

GENERAL OBSERVATIONS OF FEEDING BAGOMOLASSES TO CATTLE

At the outset, it was found necessary to conduct a number of preliminary feeding trials to fix the maximum quantity of bagomolasses which could be fed with ease and without inducing any ill-effect in the animals. At first, the animals showed a good appetite for the semi-dry bagomolasses, which, however, gradually waned and after about two to three weeks the animals simply licked and nibbled the food intermittently. It was eventually observed that bagomolasses could be fed for a longer time, if it was soaked in water before offering to the animals. Though this procedure offered good promise, it was later found that even after soaking the consumption of bagomolasses was not high enough to warrant its inclusion in a ration. The next step followed was to feed bagomolasses mixed with a bulky concentrate such as wheat bran. The mixture, as before, was fed after soaking. This procedure was quite successful and the animals could be

induced to eat 4 lb. of bagomolasses without any difficulty. As feeding of bagomolasses was found to be accompanied by excessive diuresis, other points of importance recorded in this connection were, that the animals must have free access to common salt and more frequent watering than usual. Diuresis in cattle has been noted by Warth [1926] and recently by Sen, Ray and Talapatra [1942] in animals fed on paddy-straw. According to these authors, diuresis is induced by the large quantity of potassium salt present in paddy-straw. Cane-molasses is also rich in potassium (about 2·62 per cent on dry basis) and it is likely that the observed diuresis is due to the high amount of potassium present in bagomolasses which contains two-third of its weight of molasses. In this connection, an experiment was designed to study the effect of withdrawal of salt from the ration containing bagomolasses on the general condition of experimental animals. The effect of the withdrawal of the salt was quick and marked. Within two weeks of salt-starvation, there was a significant drop both in body-weight and food consumption. On the average, the animals lost 17 per cent of their body weight and the food consumption decreased by about 22 per cent in terms of dry matter. As soon as the animals were given free access to common salt, its consumption during the week following the complete deprivation of salt was on the average 2·6 oz. per day per animal of about 500 lb. body-weight. With progressive feeding this high consumption of salt gradually decreased to the normal consumption of 1·5 oz. per animal per day. It is apparent from the foregoing evidence that even the normal requirement of common salt in cattle is high when bagomolasses is incorporated in the ration. With the increased intake of common salt, the diuresis was noticed to be considerably reduced. Singh and Singh [1934, 1935] have recorded that when molasses was fed to working bullocks which were not given common salt, the animals often showed frothy salivation. When these animals were allowed free access to salt, the trouble disappeared completely.

Effect of incorporating bagomolasses on the consumption, digestion and utilization of a ration

Experimental

For this experiment four country bullocks, each weighing about 500 lb., were used. At first all the animals gained in weight for a short period and gradually settled down to a more or less stationary weight which averaged 550 lb. ±10 lb. In order to make a comparative study, the whole investigation has been divided into two distinct periods. In period 1, the ration consisted of wheat-*bhusa* of known nutritive value fed *ad lib.* and a fixed amount of wheat-bran of unknown nutritive value (with no bagomolasses). During period 2, the ration was changed and was made up of equivalent amount of the same wheat-bran mixed with 4 lb. of bagomolasses and the same wheat-*bhusa* fed *ad lib.* The mixture of bran and bagomolasses was fed after soaking in water. In both the periods, the animals had free access to common salt. Each test-ration fed in periods 1 and 2 was continued first for a preliminary period of four to six weeks followed by a ten-day quantitative collection of excreta and of any food residue left, for subsequent chemical analysis.

The percentage composition of the feeding stuffs used during the experiment is given in Table I. Table II shows the dry matter consumption under each feeding test. The nutrients consumed from the feeds and voided in the faeces are detailed in Tables III and IV.

TABLE I

Percentage composition of feeding stuffs

Percentage (on dry basis)

Feeding stuff	Dry matter (percentage)	Organic matter	Crude protein	Ether extract	Crude fibre	Nitrogen-free extract	Total ash	Lime (CaO)	Phosphate (P_2O_5)
Wheat *bhusa*	89·8	90·49	2·81	0·64	39·88	47·16	9·51	0·34	0·15
Wheat bran	90·0	89·58	10·37	1·33	22·40	55·48	10·42	0·22	1·91
Bagomolasses	80·7	83·91	2·71	0·37	16·33	64·50	16·09	1·43	0·22

TABLE II

Consumption per day of dry matter from different feeding stuffs

Feeding period	Consumed from: Wheat *bhusa* (gm.)	Wheat bran (gm.)	Bagomolasses (gm.)	Total ration (gm.)
Bullock 1—				
1	3,768	1,226	Nil	4,994
2	1,502	1,226	1,453	4,181
Bullock 2—				
1	2,906	1,226	Nil	4,132
2	1,246	1,226	1,453	3,925
Bullock 3—				
1	3,587	1,226	Nil	4,813
2	2,364	1,226	1,453	5,043
Bullock 4—				
1	3,859	1,226	Nil	5,085
2	1,808	1,226	1,453	4,487

TABLE III

Digestibility of whole ration in period I

Consumed from	Organic matter (gm.)	Crude protein (gm.)	Ether extract (gm.)	Crude fibre (gm.)	Nitrogen-free extract (gm.)	Total carbo-hydrates (gm.)
Bullock 1—						
Wheat *bhusa*	3,410	105·9	24·1	1,502·7	1,777·1	3,279·8
Wheat bran	1,098	127·1	16·3	274·6	679·8	954·4
Total .	4,508	233·0	40·4	1,777·3	2,456·9	4,234·2
Faecal output	2,099	135·7	21·4	814·8	1,127·3	1,942·1
Total digested	2,409	97·3	19·0	962·5	1,329·6	2,292·1
Digestibility coefficient . .	53	42	47	54	54	54
Bullock 2—						
Wheat *bhusa*	2,630	81·7	18·6	1,158·9	1,370·5	2,529·4
Wheat bran	1,098	127·1	16·3	274·6	679·8	954·4
Total .	3,728	208·8	34·9	1,433·5	2,050·3	3,483·8
Faecal output	1,719	134·1	17·2	701·2	866·5	1,567·7
Total digested	2,009	74·7	17·7	732·3	1,183·8	1,916·1
Digestibility coefficient . .	54	36	51	51	58	56
Bullock 3—						
Wheat *bhusa*	3,236	100·8	23·0	1,420·5	1,691·6	3,112·1
Wheat bran	1,098	127·1	16·3	274·6	679·8	954·4
Total .	4,334	227·9	39·3	1,695·1	2,371·4	4,066·5
Faecal output	1,964	134·9	20·3	784·5	1,024·4	1,808·9
Total digested	2,370	93·0	19·0	910·6	1,347·0	2,257·6
Digestibility coefficient . .	55	41	48	54	57	56
Bullock 4—						
Wheat *bhusa*	3,492	108·4	24·7	1,538·9	1,820·1	3,359·0
Wheat bran	1,098	127·1	16·3	274·6	679·8	954·4
Total .	4,590	235·5	41·0	1,813·5	2,499·9	4,313·4
Faecal output	2,137	147·4	22·6	848·1	1,118·9	1,967·0
Total digested	2,453	88·1	18·4	965·4	1,381·0	2,346·4
Digestibility coefficient . .	53	37	45	53	55	54

TABLE IV

Digestibility of the whole ration in period II

Consumed from	Organic matter (gm.)	Crude protein (gm.)	Ether extract (gm.)	Crude fibre (gm.)	Nitrogen-free extract (gm.)	Total Carbo-hydrates (gm.)
Bullock 1—						
Wheat *bhusa*	1,359	42·2	9·6	599·0	708·3	1,307·3
Wheat bran	1,098	127·1	16·3	274·6	679·8	954·4
Bagomolasses	1,219	39·4	5·4	237·2	937·0	1,174·2
Total	3,676	208·7	31·3	1,110·8	2,325·1	3,435·9
Faecal output	1,544	139·9	17·5	522·1	837·1	1,359·2
Total digested	2,132	68·8	13·8	588·7	1,488·0	2,076·7
Digestibility coefficient	58	33	44	53	64	60
Bullock 2—						
Wheat *bhusa*	1,128	35·0	8·0	496·9	587·6	1,084·5
Wheat bran	1,098	127·1	16·3	274·6	679·8	954·4
Bagomolasses	1,219	39·4	5·4	237·2	937·0	1,174·2
Total	3,445	201·5	29·7	1,008·7	2,204·4	3,213·1
Faecal output	1,481	133·0	16·3	474·1	837·7	1,311·8
Total digested	1,964	68·5	13·4	534·6	1,366·7	1,901·3
Digestibility coefficient	57	34	45	53	62	59
Bullock 3—						
Wheat *bhusa*	2,139	66·4	15·1	942·8	1,114·9	2,057·7
Wheat bran	1,098	127·1	16·3	274·6	679·8	954·4
Bagomolasses	1,219	39·4	5·4	237·2	937·0	1,174·2
Total	4,456	232·9	36·8	1,454·6	2,731·7	4,186·3
Faecal output	1,827	141·4	21·4	712·8	1,021·8	1,734·6
Total digested	2,629	81·5	15·4	741·8	1,709·9	2,451·7
Digestibility coefficient	59	35	42	51	63	59
Bullock 4—						
Wheat *bhusa*	1,636	50·8	11·6	721·2	852·6	1,573·8
Wheat bran	1,098	127·1	16·3	271·6	679·8	954·4
Bagomolasses	1,219	39·4	5·4	237·2	937·0	1,174·2
Total	3,953	217·3	33·3	1,233·0	2,469·4	3,702·4
Faecal output	1,621	147·8	18·7	554·9	913·7	1,468·6
Total digested	2,332	69·5	14·6	678·1	1,555·7	2,233·8
Digestibility coefficient	59	32	44	55	63	60

DISCUSSION.

Food consumption. The data on dry-matter consumption given in Table II show that the introduction of bagomolasses in period 2 had apparently depressed the total dry matter consumption in all the animals except bullock 3. When, however, the data were examined statistically, it was found that the dry matter consumption was not affected by the introduction of bagomolasses in the ration. A more interesting point in this connection is the effect of bagomolasses on the consumption of wheat-*bhusa* which was fed *ad lib.* in both the periods. When 1453 gm. dry matter from bagomolasses was introduced, the dry matter consumption of wheat *bhusa* was reduced from an average of 3530 gm. in period 1 to 1730 gm. in period 2. Thus under the present feeding condition 1453 gm. dry matter in bagomolasses had replaced 1800 gm. dry matter in wheat-*bhusa*.

Digestion. The average digestibility coefficient of the whole ration is given in Table V. The incorporation of bagomolasses in the ration has helped in the improved digestion of organic matter, which has increased from 54 in period 1 to 58 in period 2. This improvement has been effected in spite of the lowered intake of organic matter in the latter period. Briggs and Heller [1940] working with lambs have, however, shown that the inclusion of molasses in the ration invariably depresses the organic matter digestibility, although considerable differences exist in the efficiency of digestion amongst the various combinations of feeding.

Table V

Average digestibility coefficient of the whole ration

Animal	Organic matter	Crude protein	Ether extract	Crude fibre	Nitrogen-free extract	Total carbo-hydrates
Period 1						
Bullock—						
1	53	42	47	54	54	54
2	54	36	51	51	58	56
3	55	41	48	54	57	56
4	53	37	45	53	55	54
Averages	54	39	48	53	56	55
Period 2						
Bullock—						
1	58	33	44	53	64	60
2	57	34	45	53	62	59
3	59	35	42	51	63	59
4	59	32	44	55	63	60
Averages	58	33	44	53	63	60

The apparent digestion of crude protein was depressed to the extent of over 15 per cent when bagomolasses was introduced in the ration. Similar depression was also observed by other workers. Thus, in digestion study on dairy cows, Williams [1925] found that the addition of molasses to the ration had variable effects on the digestion of crude protein but in all cases the addition lowered the digestibility. Briggs and Heller [1940] also noted depression in the digestibility of crude protein when molasses was added to the fattening ration of lambs. In our experiments the animals in period 2 took relatively less of crude protein. The slightly lowered digestibility in period 2 might have been due to this relatively lower intake. But an important point to be noted in this connection is that the digestible quota of crude protein in both periods 1 and 2 had been provided by only one item of the ration, viz. wheat bran which was fed in the same quantity in both periods. Apparently, in period 2 the digestibility of protein in bran was reduced.

Several workers have noted that the digestibility of ether-extract is impaired by the addition of molasses to the ration [Perkins and Monroe, 1925; Briggs and Heller, 1940]. The reason for the lower digestibility adduced by these authors is that the large quantity of calcium and potassium salts in molasses brings about a laxative effect which is responsible for the reduced digestion of fat. In the present investigation no laxative effect in the animals was noticed in period 2. The slightly lowered ether-extract digestibility in period 2 can more easily be accounted for by the lower intake of this constituent.

The digestibility of crude fibre was practically unaffected when bagomolasses was included in the ration, while nitrogen-free extract showed a significant rise in digestion during the second period. The total carbohydrates digestibility showed a slight increase during the bagomolases feeding period.

Effect of bagomolasses on nitrogen, lime and phosphate balances

In order to assess the effect of bagomolasses on the utilization of these important nutrients balances were determined for nitrogen, lime and phosphate for periods 1 and 2. The results of the balance trials are set out in Table VI. The nitrogen-balance data show that in spite of 5 per cent lower intake and a 15 per cent lower digestion in period 2, the utilization of nitrogen when fed with bagomolasses was over 7 per cent as compared to less than 4 per cent when no bagomolasses was used. In period 2 slightly better percentage utilization was due to relatively lesser excretion of urinary nitrogen under the bagomolasses feeding.

TABLE VI

Nitrogen, lime and phosphate balance per day in periods 1 and 2

Animals	Intake (gm.)	Output: Faeces (gm.)	Output: Urine (gm.)	Output: Total (gm.)	Balance (gm.)
Nitrogen (period 1)					
Bullock—					
1	37·28	21·71	13·12	34·83	+2·45
2	33·40	21·45	11·52	32·97	+0·43
3	36·47	21·58	12·32	33·90	+2·57
4	37·68	23·58	14·05	37·63	+0·05
Nitrogen (period 2)					
Bullock—					
1	33·39	22·38	9·40	31·78	+1·61
2	32·24	21·28	8·60	29·88	+2·36
3	37·27	22·62	11·00	33·62	+3·65
4	34·77	23·65	9·00	32·65	+2·12
Lime (period 1)					
Bullock—					
1	15·51	12·09	1·34	13·43	+2·08
2	12·58	8·17	0·92	9·09	+3·49
3	14·89	10·96	1·22	12·18	+2·71
4	15·82	12·82	0·66	13·48	+2·34
Lime (period 2)					
Bullock—					
1	28·58	23·05	0·61	23·66	+4·92
2	27·72	19·90	2·54	22·44	+5·28
3	31·52	23·79	2·34	26·13	+5·39
4	29·63	23·20	1·80	25·00	+4·63
Phosphate (period 1)					
Bullock—					
1	29·07	22·91	0·11	23·02	+6·05
2	27·78	21·44	0·09	21·53	+6·25
3	28·80	21·92	0·08	22·00	+6·80
4	29·21	23·84	0·09	23·93	+5·28
Phosphate (period 2)					
Bullock—					
1	28·87	20·10	0·12	20·22	+8·65
2	28·49	21·20	0·06	21·26	+7·23
3	30·17	23·55	0·12	23·67	+6·50
4	29·33	22·76	0·10	22·86	+6·47

In regard to the lime balance, the animals showed a considerably higher retention in period 2 than in period 1, apparently due to the large intake of lime (under the bagomolasses feeding) which was practically doubled. In spite of the larger ingestion of lime in period 2 the percentage absorption was 23 as compared to 25 per cent in period 1. The phosphate intake was practically the same in periods 1 and 2 but both absorption and utilization were slightly better in period 2 than in period 1, the CaO/P_2O_5 ratios in periods 1 and 2 being 1 : 2 and 1 : 1 respectively.

DIGESTIBILITY AND NUTRITIVE VALUE OF BAGOMOLASSES

From the present experiment it has been possible to calculate the digestibility coefficients and the nutritive value of bagomolasses. The digestibility coefficient of the wheat-bran used could be determined from the metabolism data of period 1. With a knowledge of the digestibility values of both wheat-bran and wheat-*bhusa*, the digestibility coefficient of bagomolasses can be calculated by a process of elimination from the metabolism data for period 2. On the basis of

the above calculation, the digestibility coefficient of bagomolasses for each animal has been set out in Table VII. The data in Table VII indicate that the average digestibility of organic matter in bagomolasses is 69. Amongst the constituents of organic matter neither the crude protein nor the ether extract is at all digestible. The digestibility coefficient of total carbohydrates, on the other hand, is fairly high, the average being 72. From the digestibility coefficient values thus obtained, the total digestible nutrients and starch equivalent values per 100 lb. of the raw material have been calculated to be 47·0 lb. and 36·9 lb. respectively. It may be of interest to compare the total digestible nutrients and starch equivalent values of bagomolasses with those of the common feeding stuffs available in this country. This is done in Table VIII and it will be seen that the nutritive value of bagomolasses approximates that of gram husk. Bagomolasses is definitely superior in feeding value to common roughages like wheat and paddy straw.

TABLE VII

Digestibility coefficient of bagomolasses

Animal	Organic matter	Crude protein	Ether extract	Total carbohydrates
Bullock—				
1	67	Nil	Nil	74
2	64	Nil	Negligible	70
3	73	Nil	Nil	70
4	71	Nil	Nil	74
Average . .	69	Nil	Nil	72

TABLE VIII

Nutritive value of bagomolasses and other feeding stuffs

Name of feeding stuff	Digestible nutrients per 100 lb. of dry material				Digestible nutrients per 100 lb. of raw material		
	Crude protein	Total carbo-hydrates	Ether extract	Total digestible nutrients	Crude protein	Total digestible nutrients	Starch equivalent
Wheat *bhusa* . .	0·00	46·75	0·36	47·57	0·00	42·8	22·1
Wheat straw . . .	0·00	47·65	0·58	48·95	0·00	44·1	21·9
Rice straw (poor quality)	0·00	40·71	0·41	41·62	0·00	37·5	18·3
Rice straw (good quality)	0·00	49·04	0·53	50·23	0·00	45·2	29·0
Gram husk . . .	0·00	59·59	0·77	61·33	0·00	55·2	42·3
Bagomolasses . .	0·00	58·20	0·00	58·20	0·00	47·0	36·9

SUMMARY

1. Bagomolasses as a feed for cattle is quite palatable if it is fed after being mixed with a concentrate like wheat bran and soaked in water a few hours previous to feeding.

2. The feeding of bagomolasses induces a considerable amount of diuresis which can be counteracted in large measure if common salt is freely provided.

3. The incorporation of bagomolasses in the maintenance ration of bullocks does not apparently affect the digestion of any constituent excepting protein. The protein digestibility is slightly depressed.

4. The utilization of nitrogen, lime and phosphate is not affected when bagomolasses is incorporated in a maintenance ration of bullocks.

5. Bagomolasses does not contain any digestible protein or ether-extract. The digestibility

coefficient of its total carbohydrates is 72. From the values of digestibility coefficients determined, the total digestible nutrients and starch equivalent calculated per 100 lb. of the raw material are 47·0 lb. and 36·9 lb. respectively. In nutritive value, bagomolasses is thus comparable to gram husk.

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BAGOMOLASSES AS CATTLE FEED

II. THE USE OF BAGOMOLASSES FOR FEEDING WORKING BULLOCKS

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IN the U. S. A. and other countries, cane-molasses is being used for feeding cattle, particularly dairy and beef stocks. Morrison [1937], after a critical review of a large number of reports, has summarized the position as follows: When molasses is added to the ration of dairy cows, their milk yield is slightly increased due more to the higher total food consumption induced by the appetizing nature of molasses than to any significant contribution of available nutrients by the added feed. Cane-molasses is also widely used for feeding beef-cattle in the sugarcane districts of Southern States of America. It has been shown that in a ration, half of the concentrate which consists of corn and other grains can be replaced by molasses. Apparently, in such replacement, no consideration is made of the inevitable withdrawal of a portion of the digestible protein present in the grains. It has been further observed that the total quantity of digestible nutrients in molasses is 30 per cent less than in an equivalent amount of maize, so that, in feeding value, 2 lb. of molasses should be the equivalent to 1·4 lb. of maize.

In Hawaii, Henke [1933] in his study of cane-molasses as a supplement to fattening rations for swine observed that cane-molasses could replace up to 20 per cent of rolled barley in the ration for fattening pigs. A study of cane-molasses as feed for dairy cows by the same author [1934] showed that when properly supplemented with high protein foods, cane-molasses may safely be substituted for 25 per cent of other concentrates. Also, there was no indication of decreased reproductive efficiency or increase in abortion.

In Louisiana, Snell and Taggart [1932, 1933] in their study of blackstrap molasses as a feed for working mules observed that molasses was equivalent to corn grain and that it could be substituted up to 9 lb. for corn in a ration containing ground whole corn, alfalfa hay and soybean hay. Heavy molasses feeding seemed to increase sweating and 'winding' of mules.

In India, Singh and Singh [1934, 1935] have carried out feeding experiments with molasses obtained from the open-pan system of manufacturing white sugar (*Khandasari* molasses). In these experiments both working bullocks and dairy cows were used and the main conclusions arrived at were:

(*a*) 2 lb. of molasses could successfully replace 2 lb. of maize in the ration of bullocks.

(*b*) 1 lb. molasses could replace 1 lb. of bran in the ration of dairy cows without affecting the quantity or the quality of milk, and

(*c*) the replacement of the grain with molasses was possible only during winter

months; in summar, the feeding of molasses had a deleterious effect on the health of the animals.

In a later publication these authors [Singh and Singh, 1937] have further shown that as a feed for working bullocks, the factory molasses was as good as the *khandasari* variety. The conclusions drawn by Singh and Singh are, however, open to serious criticism. A careful perusal of their data shows that nutrients in the control ration of the working bullocks were significantly more than those required for maintenance and the type of work performed. The experimental animals, moreover, were adults and one could reasonably assume that the body-weights of these animals would not vary significantly even when they were fed at a higher level. Apparently, the change in body-weight, which took place in their experiment was of little value as an index to the influence of rations. It would have been quite possible for the animals to maintain the same trend of body weight, if 2 lb. of maize had been altogether absent from the control ration. As such, the replacement of the superfluous 2 lb. of maize by 2 lb. of molasses in the experimental ration cannot be regarded as evidence of the nutritive values of maize and molasses being equivalent. This, together with the fact that the total digestible nutrients in molasses are 30 per cent lower than in an equivalent amount of maize [Morrison, 1937], affords strong arguments against the conclusion reached by Singh and Singh. A further point to be considered in this connection is, how far one can justify, particularly under Indian conditions of feeding, the ignoring of the digestible protein in maize and bran, while advocating their replacement by molasses which does not contain any digestible protein.

In this communication are reported the results of an investigation carried out to throw light on the following points.

(1) Whether a molassed feed has any deleterious effect on the health and working capacity of the animals, and,

(2) Whether the molassed feed is suitable for working stock.

In order to secure information on the above points, experiments were carried out on three lines to study (*a*) the effect of the addition of bagomolasses to an inadequate village ration, (*b*) the effect of the addition of bagomolasses to the village ration enriched by a supplement and (*c*) the effect of replacement of gram husk by bagomolasses from an adequate ration for working bullocks.

CHEMICAL COMPOSITION OF BAGOMOLASSES

The bagomolasses used in the experiments mentioned above was chemically examined and the percentages dry on matter basis are given below :

Crude protein	2·64
Ether extract	0·41
Crude fibre	13·60
Nitrogen-free extract	67·02
Total ash	16·33
	100·00
Acid-soluble ash	11·04
CaO	1·25
P_2O_5	0·13

A perusal of the data given above would show that the protein content of molasses feed is low and comparable to that of a dry roughage such as wheat *bhusa*. Unlike the dry roughages, however, the crude fibre in bagomolasses is practically one-third of what is present in the former feeds. The outstanding feature in bagomolasses is the presence of a large percentage of nitrogen-free extract which is mainly composed of easily absorbable sugars. The amount of ether-extract present in molasses feed is negligible. Turning to mineral constituents, it may be seen that bagomolasses is rather rich in total ash nearly 68 per cent of which is acid soluble. In the acid-soluble ash constituents, there is an extraordinary preponderance of lime over phosphate, the former being ten times the latter. The chemical composition of bagomolasses as set out above indicates that this material cannot be put in any definite category of feeding-stuffs available in this country. Because of its low fibre-content, it cannot be considered as a roughage although it resembles a roughage in its poor protein content. For its rather large nitrogen-free extract content, bagomolasses can be assigned a place with gram or oats.

EXPERIMENTAL

Twenty-four healthy working bullocks of the Hariana and Dhani breeds were selected and divided into three groups of eight for each of the three experiments planned above. Each group was again divided into two sub-groups of four each, so that while one sub-group was receiving the control ration, the other received the experimental ration. In order to make the two rations strictly comparable, one animal of each group had a mate in the other of the same breed and

closely approximating in age, live weight, and general condition. Furthermore, to avoid any disparity in the work performed, one animal from each group was combined to form a pair for the yoke. The animals performed medium type of work, i.e. about six hours a day. The work mostly consisted of ploughing and occasionally carting. Feeding experiments were started on 2 April 1942. Towards the beginning of May, there was an outbreak of foot-and-mouth disease amongst the cattle at Izatnagar. The animals under this investigation were also affected and it was necessary to discontinue them from work. The scheduled rationing was, however, maintained, although in considerably restricted amounts. Towards the end of June, all the experimental animals recovered from foot-and-mouth disease and the routine drawn up under the different investigations was restored immediately. By the middle of September, a series of 10-day metabolism experiments was completed for the different groups of animals. The experiments continued up to 22 November 1942, when the investigation was stopped. During these experiments observations were recorded on the following points:

(1) Food consumption.
(2) Change in body weight.
(3) Working capacity and general health.

In the metabolism experiments observations were made on the nitrogen balance of both control and experimental groups of animals in each of the three lines of investigations planned. The nitrogen-balance studies were considered necessary to supply a more exact information regarding the usefulness of bagomolasses either as an added item or a substitute in the rations for working animals. During the course of metabolism experiments, some of the ainmals had to be dropped out as they became indifferent to their food on account of a peculiar sensitiveness to the metabolism harnesses. In the metabolism studies, therefore, actually 18 animals were used six for each investigation. The introduction of bagomolasses into the diet of all the experimental groups of animals was made gradually and within a short time the animals could be easily induced to consume 4·5 lb. of it.

Results and discussion

I. *The effect of the addition of bagomolasses to the inadequate village ration*

The rations for the control and experimental groups are shown below:—

	Control group	Experimental group
Crushed gram	2·5 lb.	2·5 lb.
Rape cake	1·25 lb.	1·25 lb.
Bagomolasses	Nil	4·5 lb.
Wheat *bhusa*	*Ad lib.*	*Ad lib.*
Common salt	2 oz.	2 oz.

The control ration in this experiment has been considered as a typical ration usually fed to the working bullocks in the villages of the United Provinces. A calculation of its nutritive value shows that it contains 0·5 lb. digestible protein and 4·5 lb. starch equivalent which may be considered sufficient for the maintenance of the animals of 750 lb.—800 lb. body-weight selected for the experiment. In fact the adequacy of the control ration for maintenance purpose was verified from a nitrogen-balance experiment which had indicated that all the experimental animals maintained a positive nitrogen balance. Theoretically, however, the ration was inadequate for animals required to perform medium type of work. In the experimental ration, 4·5 lb. of bagomolasses (equivalent to 3·0 lb. of molasses) has been added over and above the quantity supplied in the basic control ration. The introduction of bagomolasses has contributed an extra quota of energy of about 2 lb. This, together with the energy from the rest of the ration, supplied energy slightly more than what is theoretically required by animals doing medium type of work.

Food consumption. The total dry matter consumed by animals in the two feeding groups is shown in Table I.

TABLE I

Total dry matter consumption per animal per day in gm.

Animal No.	From inadequate village ration	From inadequate village ration + bagomolasses
1	6,325	7,886
2	6,270	6,525
3	6,305	7,186
Average	6,300	7,199

The data show that the animals receiving experimental ration containing bagomolasses apparently consumed more dry matter than the animals receiving control ration. However, this increased consumption was found on statistical analysis to be insignificant.

Live weight. The animals on control ration were found to lose weight gradually as the time of feeding progressed. Their average weight at the start of the experiment was 785 lb. and decreased by the end of the experiment to an average of 736 lb. On the other hand the animals on experimental ration maintained a more or less constant body-weight. Their average body weight at the beginning of the experiment was 801 lb. and at the end, 809 lb. However, when the change in live weight of the two groups was statistically examined the difference between the two groups was not significant.

Nitrogen-balance. The nitrogen-balance of the two groups of animals has been set out in Table II.

TABLE II.

Effect of bagomolasses on the nitrogen metabolism of working bullocks

Experiment I	Animal No.	Nitrogen intake gm.	Nitrogen excretion		Balance gm.
			In faeces gm.	In urine gm.	
Village ration	1	100·24	39·90	61·06	—0·72
	2	99·84	46·44	51·56	+1·84
	3	100·09	45·05	56·16	—1·12
Average	..	100·1	43·8	56·3	+0·0
Village ration + bagomolasses	1	108·18	41·97	45·66	+20·55
	2	98·31	32·29	45·96	+19·06
	3	103·10	38·89	43·56	+20·65
Average	..	103·2	37·8	45·1	+20·4

It is apparent from Table II that the animals on control ration were not faring too well, two out of three being, in fact, in negative balance. When bagomolasses was added to the village ration at the rate of 4·5 lb. per animal per day, the nitrogen balance rose to such high positive balance as 20·4 gm. per animal per day. This unique retention of nitrogen was observed in all the three animals. A perusal of the nitrogen-balance data shows that although the ingestion of nitrogen under the two feeding conditions was practically the same, both digestion as well as utilization were significantly higher when bagomolasses was added to the inadequate village ration. Obviously the carbohydrates in bagomolasses helped not only in the better digestion but also in sparing protein under the working condition. This observation on the increased digestibility of protein is contrary to what was found in animals at rest [Ray and Talapatra, 1945] and requires further confirmation.

II. *The effect of the addition of bagomolasses to the village ration enriched by a supplement*

The purpose of this experiment was to find out whether bagomolasses could be added with any benefit to a poor village ration which had been already enriched by a commonly available feeding stuff like wheat bran.

The rations for the control and experimental groups of animals are shown below:

	Control group	Experimental group
Crushed gram	2 lb.	2 lb.
Rape cake	1 lb.	1 lb.
Wheat bran	3 lb.	3 lb.
Bagomolasses	Nil	4·5 lb.
Wheat-*bhusa*	*Ad lib.*	*Ad lib.*
Common salt	2 oz.	2 oz.

Since the body weights of the animals in these groups were slightly lower than those of the animals in the previous investigation, a slight reduction in the amounts of crushed gram and rape cake fed to these groups was necessary to keep the digestible protein level the same as in animals in the previous lot.

In the previous experiment it had been shown that when the animals were made to work when fed on the poor village ration the negative nitrogen balance indicated a catabolism of body tissues to supply the necessary energy for working. It was considered, therefore, that when an extra amount of concentrate in the form of wheat bran, which is easily available in the countryside, is added to a poor village ration, the catabolism of body tissues can be altogether stopped or at least reduced. The addition of wheat bran increased the digestible protein supply by 33 per cent and starch equivalent by about 10 per cent. Even after this enrichment the supply of energy was 25 per cent below the normal theoretical

requirement. Since bagomolasses was an energy-giving food it was thought that the incorporation of this item even in a modified village ration would produce a beneficial result.

Food consumption. The total dry matter consumption by the animals in the two groups is shown in Table III.

TABLE III

Total dry matter consumption per animal per day in gm.

Animal No.	From village ration enriched by a supplement	From village ration enriched by a supplement + bagomolasses
1	4,605	6,195
2	5,197	5,603
3	4,979	5,780
Average	4,927	5,859

The data show that the consumption of the ration was higher (about 19 per cent) when bagomolasses was included and this higher consumption was found statistically significant.

Live weight. The live weights of the control and experimental groups showed that it was practically the same under the two feeding conditions.

Nitrogen balance. The results of the nitrogen balance are given in Table IV.

TABLE IV

Effect of bagomolasses on the nitrogen metabolism of working bullocks

Experiment II	Animal No.	Nitrogen intake (gm.)	Nitrogen excretion		Balance (gm.)
			In faeces (gm.)	In urine (gm.)	
Enriched village ration	1	94·81	32·88	51·57	+10·36
	2	98·76	34·09	47·87	+16·80
	3	97·31	40·07	46·47	+10·77
Average	..	97·0	35·7	48·6	+12·6
Enriched village ration + bagomolasses	1	101·01	35·56	47·17	+18·28
	2	97·07	32·67	47·57	+16·83
	3	98·25	29·14	42·67	+26·44
Average	..	98·8	32·5	45·8	+20·5

It will be seen that the animals receiving the control ration showed a fairly marked positive balance of nitrogen. Apparently, the poor village ration could be improved by the introduction of wheat bran. It has already been mentioned that even after the enrichment of the poor viallge ration, the energy supply was 25 per cent below the normal requirement. This apparent deficiency was compensated by the higher quota of digestible protein supplied in the modified ration. This extra protein perhaps met the energy requirement for work. When bagomolasses was added to the modified village ration there was no apparent beneficial effect. The average retention of nitrogen however increased from 12·6 gm. (on modified village ration) to 20·5 gm. (on modified village ration + bagomolasses). In this case also the higher retention was due to the combined results of better digestion and utilization.

III. *The effect of replacement of gram husk by bagomolasses in an adequate ration for working bullocks*

It has been mentioned in part I of this series [Ray and Talapatra, 1945] that bagomolasses is comparable to gram husk in nutritive value. In the present investigation, an experiment was planned to study the effect of replacement of an equivalent amount of gram husk in a theoretically adequate ration for working bullocks.

The rations for the control experimental groups of animals are as follows:

	Control group	Experimental group
Gram husk	4·5 lb.	Nil
Wheat bran	3·0 lb.	3·0 lb.
Maize	2·0 lb.	2·0 lb.
Rape cake	2·0 lb.	2·0 lb.
Ardawa	2·0 lb.	2·0 lb.
Bagomolasses	Nil	4·5 lb.
Wheat-*bhusa*	*Ad lib.*	*Ad lib.*
Common salt	2 oz.	2 oz.

Food consumption. The total dry matter consumption by the two groups of animals is shown in Table V.

TABLE V

Total dry matter consumption per animal per day in gm.

Animal No.	From an adequate ration where gram husk is included	From an adequate ration where gram husk is replaced by bagomolasses
1	8,389	8,005
2	8,136	8,257
3	7,884	8,587
Average . .	8,136	8,283

These data show that animals in the two groups consumed practically the same amount of dry matter.

Live weight. The effect of the two different feeding conditions on the live weight of the two groups has been practically the same, except that one animal on the experimental ration showed an abrupt rise in weight towards the end of the experiment.

Nitrogen balance. The nitrogen balances found in this experiment are shown in Table VI.

TABLE VI

Effect of bagomolasses on the nitrogen metabolism of working bullocks

Experiment III	Animal No.	Nitrogen intake (gm.)	Nitrogen excretion		Balance (gm.)
			In faeces (gm.)	In urine (gm.)	
Adequate ration containing gram husk	1	138·66	53·77	51·36	+33·53
	2	136·75	58·32	46·86	+31·57
	3	134·84	53·55	49·76	+31·53
Average	..	136·7	55·2	49·4	+32·2
Adequate ration where gram husk is replaced by bagomolasses	1	130·67	53·97	46·46	+30·24
	2	132·58	52·80	51·76	+28·02
	3	135·07	50·39	51·36	+33·32
Average .	..	132·8	52·4	49·9	+30·5

From the closeness of the two values of nitrogen retention it is evident that gram husk could be successfully replaced by bagomolasses.

HEALTH AND WORKING CAPACITY OF THE ANIMALS

The health of the experimental animals fed with and without bagomolasses was apparently normal. As has already been stated, all the molasses-fed animals maintained their live-weights during this short period but whether they would have done so for longer periods could only be determined by carrying out long-term experiments.

A close watch was kept on the working capacity of the experimental animals and controls. The conclusion drawn from this short-period and admittedly empirical observation is that the groups of animals receiving bagomolasses exhibited a working capacity which was comparable, if not superior, to that of their yoke mates rot receiving bagomolasses.

In spite of the animals getting a fairly large amount of salt (2 oz. a day), the urinary excretion as shown in Table VII was significantly higher in the bagomolasses groups than in the control groups.

TABLE VII

Total urinary excretion per animal per day

—	Animal	Vol. of urine excreted per day in c.c.	
Experiment I			
(*a*) Village ration at work	1	6,155	Average 5,218
	2	4,390	
	3	5,109	
(*b*) Village ration + bagomolasses at work	1	6,489	Average 6,817
	2	7,283	
	3	6,580	
Experiment II			
(*a*) Enriched village ration, at work	1	6,000	Average 4,976
	2	4,333	
	3	4,594	
(*b*) Enriched village ration + bagomolasses at work	1	6,702	Average 7,220
	2	7,831	
	3	7,128	
Experiment III			
(*a*) Adequate ration containing gram husk at work	1	5,571	Average 5,012
	2	4,290	
	3	5,174	
(*b*) Adequate ration, gram husk replaced by bagomolasses, at work	1	6,493	Average 7,283
	2	8,445	
	3	6,910	

But, since the animals maintained their live weights and general condition of health, this slight diuresis seems of no consequence.

Summary

An inadequate village ration for working bullocks can be improved by supplementing with bagomolasses without inducing any bad effect on the health. The higher retention of nitrogen in the supplemented feeding suggests that when the energy-deficient typical village ration is fortified by adequate amounts of energy-giving food like bagomolasses, the necessity for the costly protein concentrate can be considerably curtailed.

In an adequate ration of working bullocks gram husk can be economically substituted with bagomolasses without any ill-effects on the animals.

The health and working capacity of animals receiving bagomolasses to the extent of 18 per cent to 27 per cent dry matter in the ration compared well with those of the control groups during a short-term experiment.

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A SURVEY OF THE INCIDENCE OF HELMINTH INFECTION IN INDIA AT THE IMPERIAL VETERINARY RESEARCH INSTITUTE, IZATNAGAR

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As a necessary prelude to subsequent experimental work on the control of the more common and important helminth parasites of sheep, goats, cattle and buffaloes, the Imperial Council of Agricultural Research sanctioned a scheme for a survey of the incidence and nature of helminthic infections of these animals in India. A part of the scheme was carried out, from 1 November 1940 to 31 October 1942, at this Institute and a general survey of the helminthic infections of domestic ruminants in the Punjab, North-West Frontier Province and Sind was made. For the purpose of this survey, material was collected from the slaughter houses for subsequent examination and collections of helminths at the veterinary hospitals were also examined. Some specimens of worms were also received for examination through the courtesy of the veterinary field staff. In the Punjab 21 districts were visited in the summer months, while in the North-West Frontier Province and Sind only Peshawar and Karachi respectively were visited just after the rainy season. None of these places could be visited more than once.

Despite some serious limitations under which the work was carried out, an extensive survey of the incidence, intensity and nature of the helminthic infections of sheep, goats, cattle and buffaloes was made. Over ten thousand specimens of worms were examined and useful data collected. In the Punjab a total of 191 sheep, 150 goats, 58 heads of cattle and 19 buffaloes were examined. Out of these 134 (i.e. 71 per cent) sheep, 93 (i.e. 62 per cent) goats, 36 (i.e. 62 per cent) heads of cattle and 16 (i.e. 84 per cent) buffaloes were found infected with worms. At Karachi out of eight sheep, six goats and five heads of cattle examined five sheep, four goats and three heads of cattle were found infected while at Peshawar 12 out of 15 sheep and all the eight goats examined were found harbouring helminths. In Table I are indicated the number of animals of each species examined, the number found infected and the incidence of infection at the various places visited. In Table II are given the names of the more common parasites, their incidence of infection in the various species of hosts and some general remarks on their distribution.

Table I

Number of animals examined and the incidence of infection at various places

Locality	Host											
	Sheep			Goats			Cattle			Buffaloes		
	Number of animals examined	Number of animals infected	Incidence of infection (per cent)	Number of animals examined	Number of animals infected	Incidence of infection (per cent)	Number of animals examined	Number of animals infected	Incidence of infection (per cent)	Number of animals examined	Number of animals infected	Incidence of infection (per cent)
Ambala	10	8	80	4	3	75	6	5	83	—	—	—
Amritsar	8	6	75	5	3	60	—	—	—	—	—	—
Dalhousie	6	5	83·3	3	2	66·6	2	2	100	—	—	—
Delhi	14	12	85·7	6	5	83·3	4	3	75	—	—	—
Ferozepur	9	7	77·7	7	3	42·9	3	2	66·6	1	1	100
Gujaranwala	8	5	62·5	4	2	50	—	—	—	—	—	—
Gurdaspur	5	4	80	6	4	66·6	—	—	—	2	2	100
Hissar	4	3	75	3	1	33·3	—	—	—	—	—	—
Hoshiarpur	5	4	80	8	3	37·5	—	—	—	—	—	—
Jhelum	10	6	60	8	6	75	—	—	—	—	—	—
Jullunder	12	8	66·6	10	7	70	4	2	50	3	2	66·6
Kangra	10	9	90	4	4	100	—	—	—	—	—	—
Karnal	10	6	60	4	2	50	5	3	60	—	—	—
Lahore	28	22	76·6	15	11	73·3	10	6	60	8	7	87·5
Ludhiana	10	3	30	12	4	33·3	4	2	50	—	—	—
Lyallpur	6	4	66·6	10	6	60	5	2	40	—	—	—
Murree	7	6	85·7	6	5	83·3	3	2	66·6	—	—	—
Rawalpindi	6	4	66·6	14	10	71·4	5	3	60	—	—	—
Rohtak	4	2	50	—	—	—	—	—	—	—	—	—
Sheikhupura	9	4	44·4	12	6	50	—	—	—	—	—	—
Sialkot	10	6	60	9	6	66·6	7	4	57·1	5	4	80
Total in the Punjab	191	134	71	150	93	62	58	36	62	19	16	84
Karachi	8	5	62·5	6	4	66·6	5	3	60	—	—	—
Peshawar	15	12	80	8	8	100	—	—	—	—	—	—

TABLE II

Some more common parasites and their incidence of infection

Host and number of animals examined in each case

Name of parasite	Sheep 214		Goats 164		Cattle 63		Buffaloes 19		Remarks
	Number of animals infected	Incidence of infection (per cent)	Number of animals infected	Incidence of infection (per cent)	Number of animals infected	Incidence of infection (per cent)	Number of animals infected	Incidence of infection (per cent)	
Fasciola spp.	42	20	26	16	12	19	5	26	
Dicrocoelium dendriticum	19	9	10	6	4	6	—	—	In hilly tracts only
Cotylophoron cotylophorum	54	25	30	18	20	32	7	37	
Paramphistomum cervi	76	36	53	32	24	38	11	58	
P. explanatum	—	—	—	—	2	3	6	32	
Gastrothylax crumenifer	96	45	49	30	24	38	12	63	
Protostrongylus rufescens	52	24	28	17	—	—	—	—	In hilly tracts only
Dictyocaulus filaria	34	16	23	14	—	—	—	—	
Haemonchus contortus	92	43	45	27	18	29	5	26	
Mecistocirrus digitatus	—	—	—	—	14	20	2	11	
Bunostomum spp.	60	28	38	23	19	30	7	37	
Oesophagostomum spp.	43	20	30	18	11	17	—	—	

It has not been possible to collect sufficient data which could provide definite information on the incidence and intensity of helminthic infections at the different places visited and the list of parasites compiled also cannot be claimed to be exhaustive, for a wide and more intensive survey in different seasons, with better facilities, may further bring to light some more species of worms or indicate a higher incidence of infection in farm-stock. This survey has, however, revealed a very widespread occurrence of helminth parasites in sheep, goats and cattle in the Punjab, North-West Frontier Province and Sind. The data collected is likely to be of great value in the selection of important parasites for immediate investigations with a view to combating the more serious losses due to helminthiasis in farm-stock. The parasites which need immediate attention, owing to their incidence of infection and economic importance are the liver flukes (*Fasciola* spp. and *Dicrocoelium dendriticum*, the latter in the hilly tracts only), amphistomes (*Cotylophoron cotylophorum*, *Paramphistomum cervi*, *P. explanatum* and *Gastrothylax crumenifer*), the lungworms (*Dictyocaulus filaria* and *Protostrongylus rufescens*), the stomachworms (*Haemonchus contortus* and *Mecistocirrus digitatus*) the hookworms (*Bunostomum* spp.) and the intestinal nodular worm (*Oesophagostomum* spp.). Small trichostrongyles and hydatid cysts are also fairly common.

SUMMARY

A general survey of the helminthic infections of domestic ruminants in the Punjab, North-West Frontier Province and Sind was made. A very widespread occurrence of helminth parasites in sheep, goat and cattle in these areas has been revealed.

FIG. 1. Natural case. Extensive proliferation of gums and shrouding of the teeth.

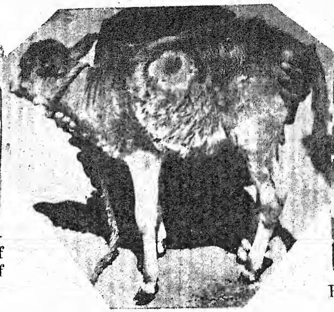

FIG. 2. Enlarged precrural glands.

FIG. 3. Natural case. Enlarged suprascapular gland.

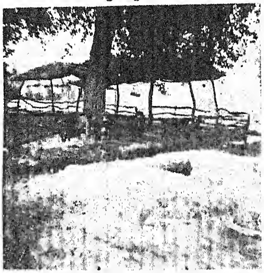

FIG. 4. A tick-proof paddock.

FIG. 5. Abomasum showing ulcerations.

FIG. 6. Liver, enlarged and petechiated.

N. B. Figs. 2, 5 and 6 are taken from a case in which the disease was transmitted hereditarily by the second generation adults of *Hyalomma aegyptium*, at the Imperial Veterinary Research Institute, Mukteswar.

CONTROL OF ACUTE THEILERIASIS IN CALVES IN THE PUNJAB

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(With Plate V)

In May 1940, we were called to attend a severe outbreak of acute theileriasis in calves of less than three months in age on a farm in the Multan district. On previous occasions the malady was recognized to have caused heavy death among calves on the farm. Locally the disease is known as *kanpher ki bimari*, i.e. illness with marked swelling of the parotid glands. It is produced by an endoglobular parasite, provisionally identified as *Theileria annulata*. The disease is prevalent from March to September, and the rate of mortality from 1936 to 1939 ranged between 13 and 23 per cent. Up to June 1940, the rate had reached 10 per cent. Since the chemotherapy of theileriasis has not yet been developed to a satisfactory stage, it is merely intended in this article to deal with the control measures adopted in endeavouring to eradicate the disease. A brief description of the symptoms as well as post-mortem lesions is given so that methods of diagnosis may be more widely known.

Symptoms

The disease occurs in acute and in subacute forms. In the former, the characteristic symptoms are dullness, discharge from the eyes and nose, raised body temperature (103°F. to 106°F.), marked swelling of the parotid, suprascapular and precrural lymphatic glands (Plate V, figs. 2 and 3), frothy salivation, constipation followed by diarrhoea, ulcerated gums (as a result of which the calves cannot suckle) and a subsequent shrouding of the teeth (Plate V, fig. 1). About the third week, most of the calves die and in the remainder symptoms become subacute. In some fatal cases, respiratory troubles, such as bronchitis and catarrh of the lungs have been observed. In the subacute form, the above symptoms are milder. Ailing calves continue to suckle. The body temperature usually remains below 104°F. Faeces are soft and yellowish in colour. Visible mucous membranes are pale and anaemic. The eyelids and the surrounding regions are oedematous. Such cases, with proper care and nursing, are likely to recover after four weeks from the onset of the disease.

It may be mentioned that gingivitis and stomatitis are the most severe complications and render the animals unable to suckle. This results in loss of condition and progressive emaciation, leading finally to death. Another and less common complication is pneumonia.

Lesions

The condition of the carcase is poor, mucous membranes are pale and in the majority of cases subcutaneous tissues, including fat, are stained deep yellow. The gums are red and ulcerated and show a light-grey gangrenous deposit. There may be pneumonia. The pericardial fat is deep-yellow and the heart muscle pale. The liver is soft and icteric, petechiated and greatly enlarged (Plate V, fig. 6). The gall bladder is usually distended and contains coagulated bile of dark-green colour. The spleen is soft and much enlarged. The urinary bladder often contains yellow coloured urine. The abomasum shows characteristic ulcerations (Plate V, fig. 5). It may be emphasized here that prognosis is favourable only in cases where the mouth lesions are not severe.

Control measures

Sen and Sreenivasan [1937] tried several drugs for the treatment of experimental cases of theileriasis in hill bulls. Of these, atebrin (Bayer) was the only one which cured as many as 55 to 57 per cent of infected cases, but they also noted that natural recovery took place to about the same extent. Since so far no drug treatment has proved satisfactory for this disease, an attempt was made to control the arthropod vector, viz. *Hyalomma aegyptium*, with which the farm was found to be extensively infested. It has been proved to our satisfaction by one of us (H. N. R.) that this tick is responsible for transmitting acute theileriasis to

young calves and that the infection is transmitted hereditarily only in the adult stage of the tick and not in the larval or nymphal stages. As found by Fotheringham and Lewis [1937], this is quite different from what has been experienced in *T. parva* infection.

The first object was to save the new-born calves. For this purpose, they were divided into two groups, viz. weaned at birth and unweaned. For the former, an area was rendered tick-proof by first digging up the earth to a depth of about 18 in. and then burning dry leaves and grass over the dug-up area. Subsequently, a moat about 9 in. wide and 6 in. deep surrounding the area was filled with water. Attendants were engaged exclusively for this tick-proof paddock. It was observed that calves, which were detained here for three months, were susceptible to infection when removed to an infected area, though none died. For the calves of the second group, more elaborate arrangements were made. Cows in advanced pregnancy were isolated from the main herd at a place about half-a-mile away. Before removing them to this segregation camp, all ticks on the animals were hand-picked, their tails and ears dipped in, and rest of the body sprayed every third day with an arsenical solution consisting of soft soap 11 oz., liquid paraffin 1 lb. $2\frac{1}{2}$ oz., sodium arsenite 1 lb. 1 oz. and water 50 gallons. Separate attendants and grazing pastures were arranged. There were also several calves which were weaned at birth, but were below two months in age. All these calves were dipped in the arsenical solution using a Cooper's portable swim-bath and housed in a paddock at some distance from the main farm. Dipping was done every third day as before. A few of these calves developed the disease, but at the first appearance of symptoms they were returned to the sick ward where they were given symptomatic treatment, along with good nursing. The floor of the sick ward was covered with hay bedding about 4 to 6 in. thick, to afford comfort and at the same time to prevent them from licking the earth.

Control by vaccination

In 1940, one of us (H. N. R.) observed that the introduction of dead parasites rendered healthy animals immune to subsequent inoculation of virulent blood containing Koch's bodies. In the light of this observation, a vaccine was prepared by making a suspension in 5 per cent formalin of lymph glands and spleen from a heavily infected case on the farm. Soon after birth, calves were vaccinated subcutaneously with 5 c.c. of this vaccine and kept in the tick-free paddock for 14 days before being transferred to the tick-infested area. Between May 23 and June 13, 1940, 19 calves (7 females and 12 males) were treated in this manner. Of these, 16 were transferred to the tick-infested area--the remaining three, being born of heavy milkers, were detained in the tick-free paddock. Some of the calves, 14-21 days after being liberated in the tick-infested area, showed a rise of body temperature, but none of them died. As a control experiment, seven calves born from June 13 and July 7, 1940, were liberated into the tick-infested area within six to twenty-five days of birth. Of these, four escaped infection and three died of acute theileriasis. In August 1940, seven calves were inoculated with a dose of 10 c.c. of the vaccine instead of 5 c.c. Five males from this batch were removed to tick-infested area, after the prescribed period, and none of these calves showed signs of the disease.

These observations on vaccination are suggestive and encouraging but, since no strict laboratory tests, such as the injection of vaccinated calves with virulent blood, have yet been made, we refrain from drawing conclusions from these few field tests.

In conclusion, we would like to stress the value of adopting tick control measures on farms where outbreaks of acute theileriasis are common amongst calves. The information so far obtained from this farm indicates that since the adoption of tick control measures, no further cases of theileriasis have been detected.

'Carrier' in theileriasis

A number of cattle in this farm showed parasites in the peripheral circulation without exhibiting clinical symptoms. Two experiments were performed on the farm to test the infectivity of 'carrier' blood to young calves of susceptible age. The results were negative. These experiments prove that the endoglobular forms of the parasite, as met with in carrier blood, represents the gametocytes and as such undergo no further development in the vertebrate host. So that development may proceed to the sporogonic part of the life cycle they must enter an intermediate host--chiefly an arthropod. Evidently, however, carrier animals are potential sources of infection to clean *H. aegyptium*. These observations

further stress the importance of tick control measures.

SUMMARY

Methods adopted for the control of acute theileriasis in calves due to *Theileria annulata* are described.

Calves less than three months of age were the most susceptible, mortality rate among such calves between 1936 and 1939 ranging from 13 to 23 per cent. The vector of the disease is tick, *Hyalomma aegyptium.*

Control measures consisted in keeping calves weaned at birth in tick-proof paddocks for at least three months.

The liberal use of tick-dip was practised both for young calves and their mothers in order to keep down the number of ticks in the farm.

ACKNOWLEDGEMENTS

Our thanks are due to the farm authorities for their hearty cooperation; to Mr Jalal Mohammad Khan, the then Deputy Superintendent of Grantee Farms in the Punjab, and to Mr Wali Mohammad Khan, M.R.C.V.S., the then Veterinary Investigation Officer of that province, for lending great assistance in carrying out our observations.

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TRANSMISSION OF PASTEURELLOSIS BY THE FLEAS (*CTENOCEPHALIDES FELIS*)

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THE general association of pasteurellosis with the seasonal flare-up of fleas has been stressed by Mehra [1943] in an article presented to the 30th session of Indian Science Congress. Sen [1925] suggested the possibility of fleas acting as mechanical transmitters of haemorrhagic septicaemia because of the morphological resemblance between the organism of human plague and that of haemorrhagic septicaemia and also because fleas are active agents in the transmission of the former from rat to rat and from rat to man. Daubney, Hudson and Roberts [1934] succeeded in transmitting the disease experimentally from mouse to mouse with *Ctenocephalus felis*. Mehra [1941], while working at this Institute, found that the disease could be transmitted from rabbit to rabbit by this species of flea. The main object of the present investigation has been (1) to confirm the findings of previous workers as regards the transmission of pasteurellosis by fleas in rabbits and (2) to test the possibility of the disease being reproduced in buffaloes through the agency of these insects.

PASTEURELLOSIS IN RABBITS

Preliminary experiments were undertaken to determine the course of bacteriaemia following an artificial infection of rabbits with *Pasteurella septica*, to find the best time for liberating fleas on the infected animal, and to determine the most suitable time for inoculating the donor rabbits. While fever is usually a reliable index of bacteriæmia, microscopical examination of suitably stained blood smears would be a more positive proof. Kraneveld and Djaenoedin [1928], working on *Pasteurella septica* infection in buffaloes have shown that rabbit inoculations are often positive when microscopical findings are negative.

In order to determine the points at issue, three adult rabbits were used. After ascertaining that their temperatures were normal, they were inoculated subcutaneously with 1·0 c.c. of a 24 hour broth culture of a virulent strain of *P. septica* (of bovine origin) at 10^{-4} dilution. Blood cultures were made at various intervals after inoculation, and at the same time rectal temperatures were recorded and blood smears stained by Leishman's method. For culture, 0·1 c.c. blood from the ear vain was taken into 9·9 c.c. of Ringer solution; from this, ten-fold serial dilutions were plated in 5 per cent serum agar. Counts were made after 24 hours at 37°C. The results are shown in Table I. Rabbits died from 14 to 18 hours after infection. Cultural and microscopical examinations gave the following results:

TABLE I

Bacteriaemia in rabbits after P. septica *infection*

Rabbit	Time after inoculation (hour)	Body temperature (°F.)	Microscopical examination	Organisms per c.c. of blood (in thousands)
1	3	102·6	Negative	0·1
	4	102·2	,,	0·2
	5	102·6	,,	1·9
	6	103·6	,,	11·5
2	7	102·4	,,	2·3
	9	105·0	,,	5·1
	11	105·2	Positive	150·0
	14	105·4	,,	3500·0
	16	101·2	,,	303000·0
3	10	104·0	,,	96·0
	12	104·0	,,	2700·0
	14	103·4	,,	30600·0

From Table I, it is seen that the organisms begin to appear in the circulation about four hours after inoculation, the bacteriaemia becoming intense after 11 hours and persisting till death. The bacteriaemia is of progressive type, its intensity being in inverse relation to the interval before death. As would be expected, microscopical examination of blood smears in the initial stages is not dependable, but the presence of bipolar organisms in smears indicates an intense bacteriaemic condition. The blood may contain before death an enormous number of bacteria, e.g. more than 300 million per c.c. Any suctorial insect feeding on such an infected animal till death would thus certainly infect itself very heavily.

Handling of fleas. Fleas were collected from local pariah dogs by combing or hand-picking. Enclosed in cloth bags surrounded by cotton wool and kept in cigarette tins at room temperature, the fleas survived for 24 to 36 hours. Those kept in flasks, with plenty of hair from dogs and pieces of filter paper, survived in a fasting condition for five days or more at 22°C. and relative humidity 80 to 90 per cent.

Before the feed, fleas were fasted for at least 12 hours in the first few experiments, the fleas were fed on small closely clipped areas of the skin along each side of the belly, both the donors and receptors being held in position under an inverted funnel of convenient size. In later experiments, however, this method was abandoned, since it was found unnecessary when the fleas were hungry. The fleas were placed on the donors when the latter were showing numerous bipolar bacteria in the blood, and were left till half-an-hour after the donors death. The infected fleas were then collected, fasted for 6 to 48 hours, and then placed on the receptors. They were kept under a funnel to make sure that they fed and were then finally allowed to remain on the receptor till death or otherwise.

Specimens of fleas which had fed on infected donors were examined at times for *Pasteurella*. For this purpose, some ten fleas were taken at random after completing the feed and were kept at 22°C. and relative humidity 80 to 90 per cent until the remainder of the batch was used for a transmission experiment. The sample fleas were then washed with alcohol, dried and ground in a mortar with saline and serial dilutions plated in serum agar. Supposed *Pasteurella* colonies were picked and tested in mice. Specks of faeces from infected fleas were examined by a similar method.

TRANSMISSION EXPERIMENTS IN RABBITS

Fifteen experiments were made in three series. In the second and third series, the fleas collected were used in successive experiments, as long as there were any fleas surviving and positive transmissions were appearing. In the first series, there was a single experiment with 250 fleas. The second series was started with 450 fleas, and fleas were used in the five experiments in batches of 450, 75, 34, 16 and 7 respectively. The third series was started with 200 fleas, and there were nine experiments, the number of fleas used being 200, 61, 25, 14, 7, 5, 4, 2, and 1. The first experiment (first series) gave negative result, although the fleas from the donor were proved by culture to contain *Pasteurella*. In the second series, the first four experiments were positive, while that with seven fleas was negative. In the third series, all experiments were positive except the last where only one flea was used.

EXPERIMENTS IN YOUNG BUFFALOES

Transmission from one buffalo to another is usually impracticable for various reasons. It is extremely difficult to collect the infected fleas from a buffalo donor after death, as they abandon the host and hide in debris or crevices in walls, etc.; and one has to depend solely on the chance of infected fleas coming out again and biting the receptor which is accommodated in the same room. The use of disinfectants has to be avoided, otherwise vectors will be killed, and it is thus difficult to avoid the possible infection of the receptor

through the donor's excreta. Secondly, to obtain an enduring and comparatively high level of bactereamia in the peripheral circulation, such as would be essential for assuring infection through a transmitting agent, is difficult with buffaloes. Rabbits however are suitable animals since they can be easily housed and handled, and when they are kept in glass containers, the infected fleas can easily be collected after death of the donor. Since the housing arrangements for the donor and receptor are separate, the possibility of infection through infective excreta of the donor can be easily avoided.

The 11 experiments carried out may be conveniently divided into three groups. All buffaloes were under one year of age.

Group I (*One experiment*). In this an attempt was made to transmit pasteurollosis from one buffalo calf to another. The donor, however, failed to show *Pasteurella* in the peripheral circulation till death. Neverthless when a rise in temperature (2°F.) was observed, 400 fleas were liberated and left till death of the animal. Fleas collected from the dead animal were negative bacteriologically. The receptor failed to show definite infection although it was left in the closed room used for the experiment and which presumably still contained infected fleas hiding in crevices, debris, etc. Discharges and other contaminated articles only were removed and no attempt was made at disinfection. This experiment cannot be considered satisfactory, since for an unknown reason, the receptor died 15 days after exposure to the fleas. A careful cultural examination of its bone-marrow gave negative results.

Group II (*Three experiments*). Rabbits were used as donors and buffalo-calves as receptors. About 500 fleas were used in each of the three experiments. They were liberated on the donors when a rise of temperature of 2°F. had occurred. The *Pasteurella*-infected fleas, after fasting for one-and-a-half to two hours, were fed on the receptors (one buffalo in each experiment) for one hour on a closely clipped circumscribed area of skin under a funnel and then finally liberated. Fleas collected from the receptor buffaloes on the following day were shown to contain *Pasteurella*. None of the three receptor buffaloes showed clinical symptoms and were liberated from the experiment a fortnight later.

Group III (*Seven experiments*). The number of fleas used in the experiments were 808, 1,600, 1,600, 160, 2,000, 340 and 3,000 respectively. As soon as *Pasteurella* were observed in the donor fleas which had been fasted for about 24 hours were allowed to feed till half-an-hour after the donor's death. In six out of the seven experiments the fleas got an infective feed for a period of nearly five hours, while in the seventh it was two hours. In one case only (experiment 2, with 1,600 fleas) was transmission successful. In experiment 5 the receptor died after three days. There was slight oedema where the infected fleas had fed, but blood smears taken after death showed no bipolar organisms. Heart blood and bone marrow examined bacteriologically also yielded negative results. The spleen was slightly haemorrhagic, endocarditis was present and also slight haemorrhagic gastritis and enteritis.

Infectivity of fleas after varying periods of fasting

Thirty-four lots of infected fleas, each containing about 10 individuals, were examined bacteriologically after fasting for 1 hour to 120 hours. Table II shows the results.

Table II

Infectivity of fleas after varying periods of fasting

Period of fasting (hours)	Total samples examined	Positive results with
1	4	2
12	2	1
16	1	0
20	3	2
24	4	3
36	2	0
40	1	1
41	1	0
43	1	1
48	2	1
58	1	0
60	1	0
64	1	0
67	1	1
72	2	1
82	1	0
84	1	0
88	1	1
96	2	0
112	1	1
120	1	0
	34	15

Out of the 34 samples examined, 15 were positive. As would be expected the proportion of fleas found infected decreases with time since the last infective meal. More samples examined within 24 or 48 hours after the infective feed were positive than later.

Excretion of *P. boviseptica* in the faeces of fleas

Eskey and Haas [1940] determined the presence or absence of infection in the fleas by inoculating their faeces into guinea-pigs. The role of infected faeces in transmission from animal to animal or from animal to man by the rubbing of faecal droplets into abrasions or wounds has been pointed out by several investigators. In the present experiments the infectivity of six faecal samples was determined by cultural examination. Three samples collected from infected fleas at the time of their second feed after 24 hours fasting were negative, while two out of three samples collected after 48 hours fasting were positive.

Experiments to determine whether infection can occur by inoculation of infective faeces through wounds arising from bites of healthy fleas

Sample of infected faeces was collected from about 200 fleas at the time they were used for a second feed after 24 hours fasting. The sample was found positive on cultural examination after it had been allowed to remain at room temperature for 24 hours. In the second part of the experiment about 100 healthy fleas, previously fasted, were allowed to bite healthy rabbit for one hour on a closely clipped area on its belly and a suspension of the infected faecal sample was then rubbed into the bites with a pestle. The movements of the rabbit were then restricted so that the area could not be licked. The rabbit died after 24 hours of pasteurellosis.

Discussion

Out of the 15 experiments using a virulent *Pasteurella* strain in rabbits, 12 gave positive results and under these conditions evidently very few fleas were necessary for transmission.

In young buffaloes, eight transmission experiments were negative, one was positive and two must be put as doubtful. Since the method employed with buffaloes was the same as with rabbits, there appears to be no reason for the failure with buffaloes unless the vectors were incapable of inoculating a sufficient number of organisms. If this is so the species of fleas used must be considered an inefficient vector. From cultural examination of infected batches of fleas which have taken an infective meal it seems likely that only a certain proportion of fleas are capable of imbibing infection. According to Eskey and Haas [1940] only 38 per cent of *X. cheopis* and 20 per cent of *D. montanus* became infected in spite of multiple infective feeds. On the other hand, Douglas and Wheeler [1943] who determined the percentage of infected fleas by cultural and histological examination report that 96 per cent of these species became infected. Douglas and Wheeler, however, consider that the efficiency as a vector of a given species of flea is not solely determined by the proportion of fleas infected but is dependent on the multiplication of bacilli in the alimentary canal of the insect. It would be of some interest to ascertain the percentage of infectivity in *C. felis* and also whether *P. septica* multiplies in its alimentary canal.

Daubney, Hudson and Roberts [1934] have shown that pasteurellosis could be experimentally transmitted in mice and rabbits. So far, the experiments recorded here corroborate their findings. Mehra [1943] reports a heavy mortality amongst rodents in a locality where an outbreak of haemorrhagic septicaemia occurred and also the presence of a large number of fleas in the burrows of rats. He further showed by experimental field investigations that the fleas transmit the disease through their bites, but if, as suggested above, the inefficiency of *C. felis* as a vector is established, there remains little or no doubt that the flea in question, which incidentally is the common flea found on cattle and young buffaloes, cannot be held responsible for outbreaks of haemorrhagic septicaemia. As an alternative vector, *X. cheopis*, the common rat flea, which has been proved to be efficient in human plague, suggests itself. However, even if this flea is proved to be equally efficient in the case of haemorrhagic septicaemia, it would be difficult to incriminate it, since this species is not normally found on cattle. And assuming that it may occasionally attack cattle, it would then have to be ascertained whether rodents carry the organisms in question. It is of interest in this connection to note that Neumann [1903] isolated a supposed representative of the *Pasteurella* group from a wild rat. Smillie [1920] also encountered

pasteurella organisms in normal rats, while Meyer and Batchelder [1926] isolated 34 strains of pasteurella from wild rats during plague eradication measures along the Pacific Coast. In India, Rajgopalan and Gopalkrishnan [1939] recorded an outbreak of pasteurellosis in white mice. There is, however, no proof that these *Pasteurella* strains were pathogenic to cattle. In absence of any direct evidence of this type, *X. cheopis* could not be accepted as a vector in haemorrhagic septicaemia. For the present, therefore, it can only be stated that *C. felis* appears to be an inefficient vector and is normally incapable of transmitting haemarrhagic septicaemia in buffaloes.

SUMMARY

Experiments on transmission of *P. septica* from rabbit to rabbit and from rabbit to buffalo were tried using *C. felis*, the common dog flea. The experiments in rabbits were positive and a very small number of fleas was effective. On the other hand, in 11 experiments with buffaloes and up to 3,000 fleas as transmitting agents eight failed completely, one was positive and two were doubtful. Since most of the experiments with buffaloes were negative in spite of optimum conditions as regards the number of fleas and high virulence of the organism, it seems unlikely that in practice *Pasteurella* in bovines is transmitted by the class of insect. *Pasteurella* was found in the faeces of fleas fed on infected rabbits and then fasted for 48 hours. The organism was also found in the bodies of infected fleas which have been fasted for at least 72 hours.

ACKNOWLEDGEMENTS

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STUDIES ON NEWCASTLE (RANIKHET) DISEASE VIRUS

STRAIN DIFFERENCES IN AMENABILITY TO ATTENUATION

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IYER and Dobson [1940] attenuated an English strain of Ranikhet disease virus. This paper is based on further work on three strains of Ranikhet disease virus carried out at this Institute.

MATERIAL AND METHODS

Three virulent strains of Ranikhet disease virus, designated as Lines i, ii and iii, received from widely separated localities in this country, were selected for study. The technique of Iyer and Dobson [1940] was used throughout. Six to twelve-months old cockerels bred on the Institute farm were used for the virulence tests. Fertile eggs for virus passage were also secured from the same farm.

RESULTS

Line i. This strain remained fully virulent after 69 successive passages, carried out over eleven months.

Line ii. No change in pathogenicity was obtained by 32 serial passages carried out over five months.

Line iii. Table I gives the results obtained with passages carried out over 12 months.

TABLE I

Virulence of egg-cultured virus

Virus	Passages	Fowl inoculation			Immunity tests		Remarks
		Total number inoculated	Number died	Number survived	Number survived	Number died	
Line ii	1—18	14	13*	1	1	—	*Died Ranikhet disease
Do.	19—28	27	1†	26	26	—	†Died other causes
Do.	29—35	14	6§	8	8	—	§Died 8 to 22 days after inoculation
Do.	36—43	18	18‡	—	—	—	‡ Died Ranikhet disease
Sub-line ii (B)	34—78	81	23‖	58	54	4	‖ See discussion

DISCUSSION

Lines i and iii did not prove amenable to attenuation after 69 and 32 passages respectively in eggs. Line ii showed no change until the 19th passage, when it appeared to have become attenuated suddenly. Though the virus retained sufficient virulence to kill chick embryos, it did no apparent harm when injected into fowls but rendered them immune to test doses of different strains of virulent Ranikhet-disease virus. These results corroborate those of Iyer and Dobson [1940] who obtained an attenuated virus capable of immunizing birds after 33 passages in one case and 14 in the other. The cause of variations in the number of passages required for attenuation is, as yet, unknown and needs further study. The attenuated virus (Line ii) obtained after the 19th passage retained its antigenic properties for nine more passages and immunized 26 of 27 birds. One bird died from other causes prior to being tested for immunity. From the 29th passage another change was noticed in the behaviour of the virus, viz. six out of 14 fowls inoculated with passages 29 to 35 died 8 to 22 days after inoculation, whereas ordinarily death occurs five days after inoculation with the virulent virus. None of these birds showed the typical clinical or post-mortem appearance of Ranikhet disease. It was noticed in individual passages that out of two fowls, receiving a similar inoculum, one succumbed and the other survived. This may have been due to the resuscitation of various latent diseases. However, from the purely experimental aspect it is necessary to accept these deaths as either directly or indirectly attributable to the test inoculation.

In passages 36 to 43 all the inoculated fowls either died or were killed after developing Ranikhet disease. A complicating factor in the interpretation of these results was the spontaneous occurrence of Ranikhet disease in the healthy stock from which our experimental birds were obtained. Further passages were therefore discontinued for some time and fresh sub-lines started later from previous passage materials stored in the refrigerator. The following results were obtained with sub-line ii (B) picked up from passage 33. Out of a total number of 81 fowls inoculated between passages 34 to 78, 58 or 71·7 per cent survived and 23 or 28·3 per cent died. These results were essentially similar to those obtained in passages 29 to 35 (*supra*) indicating the necessity of more work before this attenuated virus could be successfully employed as a vaccine. All the survivors proved solidly immune to virulent virus, except four fowls which developed paralysis after the attenuated virus inoculation and ultimately died during the immunity test. This mortality, however, was evidently the result of the primary inoculation.

SUMMARY

1. Details are given of the behaviour of three Indian strains of Ranikhet disease virus during

successive egg-passages. Two strains, Lines i and iii showed no evidence of attenuation after 69 and 32 passages respectively, while a third, Line ii, was attenuated after 19 passages.

2. From 19th to 28th passages Line ii virus had no apparent harmful effect when injected into fowls and rendered them immune to test doses of different strains of virulent Ranikhet disease virus.

3. From the 29th passage onward, the attenuated virus gave irregular results. Frequently out of two fowls receiving a similar inoculum one died and the other survived, suggesting resuscitation of latent conditions and indicating the necessity of further work.

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THE OCCURRENCE AND SPREAD OF FOWL CHOLERA IN INDIA

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Early records of poultry mortality in India are the Jessore and Madras outbreaks (1817 and 1828) mentioned by Raymond [1911] and the Calcutta outbreak (fowl cholera) reported by the Indian Cattle Plague's Commission [1871]. Until the outbreak of Ranikhet disease in 1927 [Edwards, 1928], fowl cholera was being reported as the chief cause of fowl mortality in this country, though fowl spirochetosis was also well known. Since the diagnosis of Ranikhet disease, much less prominence has been given to outbreaks of fowl cholera.

In the course of a three-year's disease survey of the greater part of this country, it was not possible to find fowl cholera infection even in a single widespread epizootic among fowls. Further, all the materials suspected for fowl cholera when tested in this section have proved negative. However, in two localities, relatively near the laboratory, limited enzootics of fowl cholera, causing insignificant mortality, were observed, and authentic strains of *Past. avíseptica* recovered.

From this survey two significant conclusions emerge: (i) that contrary to common belief, fowl cholera occurs infrequently; and (ii) that, in this country, widespread epizootics are not a feature of fowl cholera. The fact, that *Past. aviseptica* could be isolated in outbreaks from two nearby localities, led us to scrutinize the present methods employed by field workers for diagnosing the disease and to investigate the various factors affecting the viability of the organism in the suspected material during transit. The results of our investigations suggest a possibility that the disease, in its enzootic form may be somewhat more prevalent than has been indicated by our survey.

EPIZOOTIOLOGY

Little attention has been paid in this country to the epizootiology of this disease. Sporadic outbreaks have occasionally been recorded, many of which were probably wrongly diagnosed on the strength of naked-eye and microscopical examination of autopsied birds. Information regarding sources of infection and methods of spread of fowl cholera is scanty, although these constitute pre-requisites for eradication or control of the disease.

Past. aviseptica possesses low powers of resistance to inimical agencies—physical, chemical, and biological. The existence of the so-called saprophytic forms of *Pasteurella*, found in soil, has been questioned [Hutyra, Marek, and Manninger, 1938], and soil can hardly be regarded as a reservoir of infection. During field observations on spontaneously-occurring fowl cholera, Pritchett, Beaudette and Hughes [1930] found healthy pullets, which were survivors from a previous outbreak, to be the foci of infection as they carried the organisms in the upper respiratory tract. Other workers [Smith, 1891-92; Klein, 1906; DeKruif, 1922; Webster, 1924,1; Smith, 1927] have also isolated *Pasteurella*

organisms, both virulent and avirulent, from the mucous membranes of apparently healthy animals. Hughes and Pritchett [1930] could not produce the disease by the oral administration of virulent *Past. aviseptica* in capsules and found that the usual channel of infection was through the upper respiratory tract. Webster [1924, 1 and 2], working with rabbit pasteurellosis, found that preceding an outbreak, there was a fall in resistance due to adverse environment, and a corresponding rise in the number of the organism which ultimately led to an outbreak of the disease. It would appear from the existing work that the more resistant are not affected, those less resistant become carriers, while hypersusceptible birds die of infection.

Experimental

An outbreak of fowl cholera provided the opportunity to study certain epizootiological aspects of the disease. This occurred in the Institute farm which is self-contained as no eggs, chicks or fowls are brought from outside, and every possible precaution is taken to prevent the introduction of diseases. The disease broke out in 1940 in a group of 40 cockerels causing the death of six only. The source of infection was undetermined. A further outbreak occurred in two adjoining pens in mid-summer, 1941, with sporadic cases over a period of a month. Out of a flock of 2,000 birds, only 11 died.

The organisms recovered were of the 'blue colony' type [Hughes, 1930] associated with enzootic fowl cholera. The recurrence of the disease on the farm, the type of organisms recovered, the insignificant morbidity and mortality and the course of the outbreak led us to suspect the existence of carrier fowls in the flock, such a possibility being in accord with the findings of Manninger [1921], Van Es [1937] and Shook and Bunyea [1939]. Therefore, soon after the second outbreak, it was decided to examine the flock for the presence of carriers.

Methods

(1) *Agglutination test*. The rapid whole-blood, stained-antigen method, employed by Shook and Bunyea [1939], was tested using serum from fowls and rabbits previously immunized with *Past. aviseptica*. The serum had a titre of 1/320. Crystal violet-stained antigens of homologous and heterologous strains were used. The reaction was unsatisfactory and the results were not clear-cut. However, the strains of fowl cholera used by us were perhaps not quite suitable for the test.

(2) *Bacteriological*. Webster [1924, 1] working with rabbit pasteurellosis, and Pritchett *et al.* [1930] working with fowl pasteurellosis, recovered the organisms from carriers by culturing the upper respiratory tracts.

Preliminary tests were conducted to standardize the technique. The following methods for obtaining material from nasal passages of fowls were compared: (*a*) nasal washings obtained by syringing the nose with saline or broth; (*b*) swabbing the nasal cavity with dry cotton swabs; and (*c*) swabbing the cavity with a wet cotton wool swab. Nasal washings invariably gave a profuse growth of contaminating bacteria. Swabs and especially wet swabs gave better results and isolated colonies of *Pasteurella* could easily be obtained.

With regard to culture media, Webster and Baudisch [1925] showed that the growth of *Pasteurella* was more profuse under a reduced oxygen pressure and on media with a trace of rabbit blood or an iron compound with strongly catalytic properties. Schütze and Hassanein [1929] also got better results by adding small amounts of blood or sodium sulphite. A test was therefore run to compare routine laboratory media and certain other enriched media. The following were used: (*a*) nutrient agar, (*b*) plain broth, (*c*) agar with 5·0 per cent ox blood, (*d*) agar with 1·0 per cent ox blood (*e*) agar with 0·5 per cent sodium sulphite, (*f*) agar with haemolyzed rabbit blood, (*g*) Fielde's medium, and (*h*) peptone broth with 10·0 per cent inactivated ox serum.

Serum broth gave thicker growth than plain broth. The counts on nutrient agar were significantly lower. All the other enriched media gave uniformly good results, with little difference in counts. As to the relative value of liquid and solid media, swabs from the nasal cavity were sown as follows: (*a*) into a tube of broth, which was incubated for 24 hours at 37°C. and then plated, (*b*) into a tube of broth, which was incubated for one hour and then plated, and (*c*) on to a blood-agar plate. Initial incubation in the liquid medium invariably led to an overgrowth of contaminants. Direct sowing on blood-agar gave discrete colonies from which *Pasteurella* colonies could be easily picked.

SURVEY OF FLOCK FOR CARRIERS

The first test for carriers was conducted soon after the second outbreak, which lasted from May 18 to June 13, 1941. Between August and December, cultures were made from the nasal passages of all fowls in the affected pens as well as the two adjoining pens. In all, 273 fowls were examined, 11 of which (4·06 per cent) yielded *Past. aviseptica* on culture. The organisms were typed and studied in detail.

Morphology, etc. In morphology, staining, colony form, etc. the organisms conformed to the genus *Pasteurella trevisan*. The 11 strains were identical in cultural and biochemical characters. All were of the ' blue-colony ' type [Hughes, 1930], produced turbidity and a heavy viscid sediment in broth, thin glistening white growth on plain agar, viscid varnish drop-like growth on blood-agar and no growth on MacConkey's medium. Gelatin was not liquified, methyl-red and Voges-Proskauer reactions were negative, and no change was produced in litmus milk. Ammonia, indol and catalase were formed and nitrates were reduced. Acid was produced in dextrose, sucrose, laevulose and mannitol. Lactose, glycerol and rhamnose were not fermented.

Serological. Tables I and II show the results of agglutination reactions. More than two serological groups are suggested. One strain (No. 574) could not be classified as it failed to produce agglutinins on repeated inoculations.

TABLE I

Agglutination titres of stock Past. aviseptica *serum against 11 carrier strains*

Serum	Homologous	Antigen										
		104	861	947	2027	654	574	836	509	102	56	95
Stock *Past. aviseptica*	1 : 640	1 : 640	1 : 640	1 : 640	1 : 640	1 : 640	1 : 640	0	0	0	0	0

TABLE II

Cross agglutination tests

Immune serum	Antigen										
	104	861	947	2027	654	574	836	509	102	56	95
104 . . .	1 : 320	1 : 320	1 : 320	1 : 320	1 : 320	0	1 : 20	0	0	0	0
861 . . .	1 : 320	1 : 320	1 : 320	1 : 320	1 : 320	0	1 : 80	1 : 160	0	0	0
947 . . .	1 : 160	1 : 320	1 : 320	1 : 160	1 : 160	0	1 : 80	1 : 160	1 : 20	0	0
2027 . . .	1 : 320	1 : 320	1 : 320	1 : 320	1 : 320	0	0	0	1 : 20	0	0
56 . . .	0	0	0	0	0	0	0	0	0	1 : 640	0
574 . . .	0	0	0	0	0	0	0	0	0	0	0

Pathogenicity. The virulence of freshly-isolated carrier strains for fowls and rabbits is shown in Table III. The organisms were grown on blood-agar for 24 hours, and washed off with serum-saline. One c.c. of this bacterial suspension standardized to contain about 200 million bacteria per c.c. was inoculated subcutaneously. The period of observation extended over seven days. Table III shows that the strains varied considerably in their virulence.

Persistence of carriers. Carriers were found in all four pens examined, although the disease appeared to have been confined to two pens only. Since the test, all birds dying on the farm have been autopsied and the materials cultured for fowl cholera with negative results. As no further outbreak occurred a part of the flock was tested for the persistence of carriers. Twenty-one months after the first examination, in March and April, 1943, cultures were again made from the nasal cavities of 287 birds. In this test, the birds were selected at random from different groups in order to obtain an index of infection for the whole farm. An entirely

TABLE III

Virulence tests

Cultures	Result of inoculation	
	Fowl	Rabbit
574	S	S
836	S	S
102	S	S
56	S	S
95	S	S
861	D in 24 to 42 hr.	D in 18 hr.
2027	Do	Do
104	Do	S
947	S	D in 18 hr.
654	S	Do
509	S	Do

NOTES.—S=Survived. D=Died.

In all cases where deaths occurred, *Past. aviseptica* was isolated.

new lot of birds was examined. Four fowls (1·4 per cent) were found to harbour the infection of the 'blue-colony' type. As the previous test gave 4·06 per cent of carriers, it would appear that the conditions on the farm had been inimical to the propagation of the micro-organism and conducive to maintenance of an adequate level of resistance in the flock.

DISCUSSION

From the above results, it can be seen that carriers could be detected 21 months after the last recorded death from fowl cholera. Though the possibility that the carriers might have originated from an entirely fresh introduction of the disease is not ruled out, it is equally possible that the condition may have been present during the whole period since the last infection. This existence of carriers explains the perpetuation of infection in apparently clean farms. Efforts at prevention and control cannot therefore be expected to succeed, if traditional measures, such as the disinfection of poultry houses, etc. and isolation and killing of diseased individuals (clinical cases), are solely employed. Elimination of carriers is the only reliable method of control. If the ratio of the carriers to clinical cases is high, such preventive measures will only give a false sense of security. The recovery from apparently healthy individuals of virulent *Pasteurella*, capable of killing fowls, constitutes a serious potential danger to the flock. The position is summed up by Van Es [1937]: 'In the light of present day evidence it appears that the crux of the problem (of fowl cholera) lies with the recognition and elimination of the healthy infection carrier'. Pritchett *et al.* [1930] concluded from their work that such a procedure was both effective and practical.

SUMMARY

A three-years' survey of the incidence of fowl cholera in India suggests that fowl cholera occurs far less frequently than is usually believed and that widespread epizootics are not a feature of the disease.

In one outbreak, the disease was observed to run a typically enzootic course. The presence of carriers to the extent of 4·06 per cent among apparently healthy fowls was established and they were even found in seemingly unaffected pens.

Pathogenicity tests showed that about one-quarter of the strains of *Pasteurella* isolated from carriers were virulent for the fowl and nearly one-half for the rabbit. Examination of another lot of fowls from the same farm, 21 months after the last clinical case of fowl cholera, again revealed the existence of carriers to the extent of 1·4 per cent.

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ANIMAL NUTRITION PROBLEM IN BENGAL

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In order that the position of animal nutrition in Bengal may be properly judged, it is necessary to realize the limitations forming the special feature of this province. Bengal presents a melancholy spectacle in that its food output whether for man or beast is as much deficient in quality as in quantity. Of the total cropped area of nearly 30 million acres (net cropped area 23 million acres), only about 0·1 million acres are under fodder crops. Even of this nearly half is in one single district. viz. Mymensingh. In other words the area in the remaining 26 districts is so small that its effective value is negligible. Of other Indian provinces, we find that the Punjab has five million acres for 15·8 million cattle, Bombay 2·5 million acres for 9·96 million cattle and U.P. 1·48 million acres for 32 million cattle. In contrast with these, the only practical expression Bengal has given as a mark of her cattle conscience or reverence for the cow is the meagre allotment of a nominal acreage of fodder (as stated above) and of the residues (straw) left over after the harvest of rice crop from 22 to 23 million acres, giving an estimated yield of 400 to 500 million maunds of straw. Even if the whole of it is available (it is well known it is not, as a part of it is used as matting, a part as thatching, etc. and a part is wasted) it will not be able to provide more than 2·5 to 3 seers of straw per head of adult population leaving out of account the young stocks.

In the case of concentrates the position is not better. The area under rape and mustard is 0·76 million acres, *til* (sesame) 0·18 million acres, linseed 0·19 million acres. The area under rape and mustard has gone down considerably, as in 1911-12 it was 1·32 million acres, There was a slow decrease up to 1920-21 after which there was an abrupt shrinkage. The present area (764,000 acres) is about three-fifths of the area 30 years ago. The by-products available from these and from wheat (0·17 million acres), barley (0·12 million acres), maize (0·10 million acres), gram (0·41 million acres), and a sprinkling of *jowar* and *bajra* all taken together do not provide more than a *chatak* per head per day.

It is not known what the exact areas are under pulses. The crop report states that the area under other food grains including pulses and *marua* is 1·3 to 1·4 million acres. To these may be added the variable by-products of rice manufacturers and a small supply of imported cakes as well as by-products from imported oilseeds and pulses.

It is difficult to ascertain from the crop reports the amount available after meeting exports and imports. It appears from earlier reports that about 3 to 4 million maunds of linseed were imported and about the same amount exported from Calcutta. Regarding wheat, rape, mustard and pulses, there is apparently a surplus of imports. Little is known about the import and export of oilcakes and grain by-products.

Nevertheless some amount of cattle feed is available from these different avenues of import; but taking the province as a whole the position is that except for an insufficient supply of a coarse fodder like rice straw and a limited supply of concentrates, we have hardly anything to offer to our cattle. Our green fodders occupy such a meagre area that not more than 0·4 million of our cattle can derive nourishment from it. Yet these constitute the sources through which we have to feed not only the very large cattle population but also other animals.

According to the census conducted in 1940 the number of live-stock in Bengal is as follows:

Cattle	22,623,367
Buffaloes	1,076,266
Sheep and goats	6,545,950
Horses and ponies	77,398
Mules	175
Donkeys	905
Camels	93
Pigs	159,743
Fowls	25,931,999
Ducks	5,055,661

It will thus be seen that our problem is not merely

a case of deficient supply but of the barest need of subsistence. The matter is further complicated on account of the fact that the feeding of rice straw is followed by certain complexities in which almost all the essential nutrients suffer from faulty assimilation.

THE GENERAL FEEDING VALUE OF RICE STRAW

A short-term feeding trial conducted at Karnal (Punjab) suggested that rice straw when fed with concentrate was superior to wheat straw and was as good as *jowar* (sorghum). Later on when work on a more comprehensive scale was undertaken in Bengal, it was revealed that under rice straw feeding not only the assimilation of more essential nutrients like protein, lime, phosphorus and potash was generally interfered with, but in some regions, specially in saline tracts, even other minerals suffered from disturbed equilibrium. The Animal Nutrition Section, Bengal, started under the grant of the Imperial Council of Agricultural Research, carried out these experiments when the present author was in charge of it. The work was directed towards the examination of the existing food sources in order that an economic formula of rationing might be devised in due regard to quality and quantity of feed. The first step towards it was to study how these foodstuffs behaved within the animal system and what were their specific characters for or against proper nutrition. The work followed broadly on two specific lines, one in reference to the direct practical problems and the other of more technical nature mainly arising as a corollary towards the solution of many such problems. This note deals with the former aspect.

Protein. It was found that when the straw was fed alone, there was an uneconomic wastage of protein fraction which was least digested (remaining largely on the negative side), but when supplemented with cake or concerntrates, the digestibility and assimilation of protein were remarkably increased. This was also the case with the bulk of organic nutrients.

Phosphorus. In the case of phosphorus the actual content is initially low, and even this is of doubtful availability.

Lime. In the case of lime, although the amount ordinarily present is apparently more than adequate from maintenance standpoint, the level necessary for ensuring a favourable or positive equilibrium is much higher than in many other straws and green fodders. There appear to be four factors responsible for this complication, viz. (1) presence of oxalic acid and possibly other allied substances which prevent the solubility of lime, without which assimilation cannot proceed, (2) high fibre content, (3) high potash content and (4) complete absence of vitamin.

Potash. As straw contains a very large percentage of potash, a very large potash ingestion (far more than is necessary) is an inevitable accompaniment of rice straw feeding; but the puzzling factor is that, in spite of such heavy ingestion, more potash is excreted through faeces and urine than is supplied in the feed. The result is that when the data are measured on a balance sheet, a negative balance for potash is recorded. In actual fact the excess potash is drained out from body resources. There are indications that in this phenomenon the nature and quality of cake and possibly other concentrates play a significant role. Thus when the straw was fed with linseed cake, the attainment of a positive balance between intake and outgo was a usual feature, but when mustard cake (which is the main concentrate in these parts) was fed, there was a general tendency to a negative balance in spite of the fact that the potash ingestion was distinctly higher (in some cases 60 per cent over the other). In a recent trial the combination of *til* or sesame cake has also exhibited a similar feature.

Soda, chlorine and magnesia. So far reference has been made to the more essential nutrients such as lime, phosphorus and potash which have recorded disturbed equilibrium under rice-straw feeding. It has since been found from more recent work that the straws specially from saline belts (and in some cases from non-saline areas also) have in addition new complexities. Thus, with Barisal straw (from saline tract) chlorine recorded negative balance in spite of feeding common salt, while in the case of another saline tract of Diamond Harbour (District 24 Parganas), magnesia, soda and chlorine all exhibited adverse equilibrium in spite of the fact that all these components were more than adequately provided. It is just possible that some of these features are endemic in respect to certain regions.

Proper utilization of straw. The straw while serving as a roughage, its main function is to supply energy. Here also the most efficient utilization occurs when it is supplemented with cake. It is a fact that an unbalanced food is

not properly utilized, and since the straw is highly unbalanced, it also behaves similarly unless it is fed with more nutritious supplement. Results have shown that weight for weight the digestibility and energy efficacy of straw is liable to be reduced to half, while protein metabolism is severely upset when the straw is fed alone. On the other hand the addition of cakes and concentrates does not only arrest such a wasteful process, but operates in a highly economic way in stimulating maximum utilization of energy, protein and other nutrients.

Varieties and strains of straw. According to the statistics on crop report, the rice in Bengal has been divided into three varieties, viz. *amon* or winter rice, *aus* or autumn rice and *boro* or spring or summer rice. In actual fact, although *amon* mainly belongs to transplanted variety, there are broadcasted and deep water varieties also. The trials so far conducted in Bengal were with the straw from transplanted *amon*, broadcasted *aus* and transplanted *boro*. Time and facilities were both limited to permit wide scale work embracing all soil and climatic belts. From the results so far obtained it appears that the energy output of all straws is more or less similar, but in many other respects each is markedly different. Thus lime requirement which is generally high under all kinds of rice straw feeding, is comparatively less with *amon*, intermediate with *aus* and highest with *boro* in which the quantity of oxalic acid is also very high.

The earlier work was mainly done with straw from Dacca and Nadia, and it was tacitly assumed that the characteristic features of straw from different soil belts would probably be the same. Thus while lime, phosphorus and potash presented a picture of complex metabolism, on feeding of straws from other tracts, in the case of straws from the saline belts of Barisal and Diamond Harbour, it was found that other components such as magnesia, soda and chlorine present similar complexities. It has been further found that in the *amon* varieties, the amount of oxalic acid is lowest in Barisal straw, considerable in Diamond Harbour straw and heaviest in Chinsurah straw. The results suggest that (1) soil condition and the environment exercise a profound influence on general as well as some specific characters of the straw grown in an area, and (2) in order to get dependable results, feeding and metabolic tests should be conducted with local animals under local conditions of feeding.

It should be stated that so far the work was done with mixed straws as were obtained from the different localities, but comparison will be more valid and helpful if such work is conducted with straw of pure strains grown in the different soil belts. Here it will be relevant to refer to some experiments conducted with pure strains, viz. *indrasail* and *lattisail*. The point of interest lies in the fact that although chemical analyses did not reveal any marked difference in their composition, nutritional tests definitely established the superiority of *indrasail* over *lattisail* straw. Not only does it emphasize the need of conducting work with other strains but also with strains from typically different soil belts.

LIME ASSIMILATION UNDER RICE STRAW FEEDING

Rice straw contains an appreciable amount of oxalic acid which has the property of keeping lime in an insoluble form. This substance, which in a soluble form is also a poison, has been also found to vary according to soil and climate as reflected in a high percentage in Chinsura straw and lowest in Barisal straw. In the case of *boro* variety of rice straw as well as in *amon* variety of Chinsurah straw oxalic acid is found in such a large quantity that it is theoretically capable of rendering insoluble not only the entire lime present in the straw but it is also potentially able to render infructuous the efficacy of lime from other food sources. A striking feature however is that a large part of soluble oxalate in the straw seems to undergo decomposition in the rumen of cattle. By this, the directly harmful effect of this substance is minimized but the possibility of some direct absorption also exists with its harmful effect. Apart from this the presence of other (still unidentified) interfering substances against lime assimilation has also been detected. A large amount of potash as found in rice straw is another factor causing disturbance in lime metabolism.

Both potassium and oxalic acid have loomed large in the recent investigations with rice straw feeding. As a matter of fact their effect on metabolism is not merely of theoretical interest, but has a far-reaching practical significance. Evidences suggest that where oxalic acid is present in large quantities, the potash content too is high. The soluble oxalates exist chiefly as potassium oxalate. In the rumen of cattle the oxalate undergoes decomposition with the production of carbonates and bicarbonates with which

potash forms new compounds and exercises such influence on the acid-base equilibrium of body fluid that the urine of rice straw feeders is invariably alkaline. This also indirectly reacts on the lime assimilation as alkaline reaction is unfavourable to the maintenance of optimum solubility of lime, without which its assimilation is retarded. A further complication lies in the fact that although a very heavy potash ingestion follows rice straw feeding, more potash is excreted through faeces and urine than is provided in the feed. This phenomenon seems to be related also with the kind and quality of cake, as in the investigations conducted linseed cake had a beneficient effect, but mustard cake had not. At present there is not sufficient information explaining the excess drain of potash from the body.

Pre-treatment of rice straw

In order that the removal of these substances could be efficiently carried out several methods have been tried. The straw was subjected to the treatment of lime, caustic soda and simply water, by keeping it soaked in the respective liquids for about 24 hours. After this period the straw was washed and fed to animals. The results suggest that while caustic soda treatment is most efficient, its cost is a serious factor against its adoption. Lime treatment involves much less cost, but water treatment has the merit of simplicity, is least costly and is able to remove 90 per cent of potash. Moreover, the loss of organic matter which is appreciable under caustic soda treatment is the least under water treatment. Yet by this simple procedure, the feeding quality of straw is improved to such an extent that protein and minerals are better assimilated and even the otherwise high lime requirement under rice straw feeding is appreciably reduced.

Feeding of concentrates with rice straw

As mustard cake forms a very important source of concentrates in Bengal, various trials were conducted with this feed in combination with rice straw. The results have been generally satisfactory with protein and many other components. But, as already stated, the excess drainage of potash under its combination is an unsatisfactory feature for which the cause still remains to be ascertained. Meanwhile tentative remedies lie in giving a better combination in which the feed should be supplemented with a number of concentrates such as linseed or/and other cakes, pulse by-products, green forages, etc. This will ensure the supply of essential proteins as absence of these ingredients from the diet is likely to bring about a corresponding breakdown of body proteins, and as cell tissues (which are composed of proteins) also contain potash, an excess drainage of the latter will be avoided by feeding rice straw with other concentrates than mustard cake.

Rice-kura and rice by-product

The rice by-product of good quality is half as rich in proteins as cakes; it contains a large quantity of high quality oil and is exceptionally rich in phosphorus, but a considerable part of this phosphorus is in a complex combination chemically known as phytin which is not generally assimilable. A collection of sample from different sources was made and analysed. It was found that the product consisted of a variable mixture ranging from pure husk to diverse grades of intermediate products. This is mainly due to the various processes used in the production of rice and rice by-products. In the manufacture of rice whether by country process or mills, the nutrients are ultimately distributed between rice, broken rice and husks. Since rice and broken rice go for human consumption, the ingredients necessary for cattle food have to be derived from the residual nutrients left in the husk. The husk produced by the country process and smaller mills are obtained in a mixed condition and form approximately 25 per cent of parboiled paddy of which little more than one-third, i.e. 9 per cent are capable of being separated as finer material.

The husks from Marshalls machine are broadly obtained at two stages, viz. *ara* and *chanta*. The *ara* husk is too coarse and contains a very large proportion of fibre and silica, whereas the *chanta* husk is finer and forms 21·25 per cent of parboiled paddy. By careful fractionation about half of it can be separated into more suitable cattle food. The main point to be noted in reference to the distribution of nutrients is that the bulk of protein and starchy parts go to rice, while oil (ether extract), soluble ash, phosphorus, potash and magnesia go to rice by-products. Both rice and rice by-products are particularly

poor in lime, but finished rice is poorer still having one-fourth of the percentage in *kura*. Of the total phosphorus in rice and its by-products as much as 51 to 94 per cent consist of phytin. Several processes for stimulating its better utilization were tried but the work could not be pursued further due to the retirement of the author from service. Work on this highly important and interesting problem deserves further attention.

SUMMARY

The limitations forming the special feature of Bengal with regard to the problems of animal nutrition have been discussed.

The general feeding value of rice straw has been dealt with.

Since the straw is highly unbalanced, it is not properly utilized unless it is fed with more nutritious supplement.

The soil condition and environment exercise a profound influence on general as well as specific characters of the straw in an area.

In order to get dependable results, feeding and metabolic tests should be conducted with local animals under local conditions of feeding.

Rice straw contains an appreciable amount of oxalic acid which has the property of keeping lime in an insoluble form. This substance has been found to vary according to soil and climate. A large amount of potash as found in rice straw is another factor causing disturbance in lime metabolism. In order that removal of these substances could be efficiently carried out several methods have been suggested.

Mustard cake in combination with rice straw gave satisfactory results as regards protien and many other components. But the excess drainage of potash under its combination is an unsatisfactory feature. An excess drainage of potash will be avoided by feeding rice straw with other concentrates than mustard cake.

Preliminary work on the utilizability of rice-*kura* and rice by-products as cattle feed was also undertaken.

THE SUSCEPTIBILITY OF DUCKS TO NEWCASTLE (RANIKHET) DISEASE

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NEWCASTLE (or Ranikhet) disease has been reported in a variety of birds, both domesticated and wild, but is most common in fowls. Workers in India and other countries have reported that ducks are susceptible to the virus [Doyle, 1927; Farinas, 1930; Cooper, 1931 and Picard, 1934]. Cooper, however, stated that 'although occasionally ducks became affected by artificial inoculation and the virus could be recovered from them, those birds were found to be relatively highly resistant'. Dobson [1939] is apparently the only worker to have reported that ducks are resistant to both experimental and natural infection. The writer has frequently heard from breeders and disease investigation officers in this country that ducks in infected localities have remained free from the disease. In view, therefore, of the diversity of opinion regarding the susceptibility of ducks to the Newcastle virus, the writer*, decided to inaugurate a series of experiments.

* This work was carried out at the Ministry of Agriculture, Veterinary Laboratory, Weybridge, England, under a scheme financed by the Imperial Council of Agricultural Research, New Delhi.

EXPERIMENTAL

Mature Pennine ducks and ducklings were used. Fowl-passaged Newcastle virus, prepared by suspending in 1·0 per cent saline a mixture of infected liver and spleen in equal quantities, was used for subcutaneous inoculation. One c.c. of this suspension contained one million M.L.D.

The experimental birds were kept individually in coops during the entire period of observation.

RESULTS.

Experiment I (Table I)

Transmission experiments. (1) On 10 November 1939, two ducks were injected each with 1 c.c. virus. As no reaction occurred, they were injected 26 days later with 10 c.c. virus. They remained healthy. To complete the observation, they were bled for serum and killed 40 days after the first injection.

(2) On 17 November 1939, two ducks were exposed to infection by placing them in a contaminated coop. They remained healthy though fowls had previously died of the infection

in the coop, and, on the 19th day, the infection was implemented by injecting them each with 10 c.c. virus. As they showed no reaction they were bled and killed 33 days after the first injection.

(3) Two more ducks were injected with 10 c.c. virus on 21 November 1939. One of these died as a result of helminthic infection on the 14th day, while the other showed no reaction and was reinjected with 10 c.c. virus on the 15th day. It remained well and was bled and killed on the 29th day after the first inoculation.

(4) Two eight weeks-old ducklings were injected each with 1 c.c. virus. They showed no reaction; on the fifth day one was killed and an emulsion of its liver and spleen was subinoculated into a fowl to test the infectivity of the tissues. The fowl remained healthy and was later shown to be susceptible to Newcastle virus infection. The tissues from the duckling, therefore, contained no virus capable of infecting the fowls.

The second duckling also showed no reaction to the inoculation and was killed on the 20th day. The autopsy of this bird revealed nothing unusual.

TABLE I

Inoculation of Newcastle disease virus into ducks and ducklings

Experiment	Birds and number	Date of inoculation	Dose of fowl virus suspension	Result
1	Duck 507 and 443	10-11-39	1·0 c.c.	No reaction
		6-12-39	10·0 c.c.	No reaction, bled and killed 20-12-39
2	Duck 633 and 764	17-11-39	Coop contact infection	No reaction
		6-12-39	10·0 c.c. subcut	No reaction, bled and killed 20-12-39
3	Duck 633 and 384	21-11-39	10·0 c.c.	Duck 633 died from other causes; no reaction in duck 384
		6-12-39	10·0 c.c. into duck 384	No reaction, bled and killed 20-12-39
4	Duckling 1 and 2	21-6-40	1·0 c.c.	No reaction. Duckling 1 killed 5th day, inoculated organ suspension into a fowl and into fertile 11 day incubated hen eggs with negative results; duckling 2 killed 20th day, nothing unusual.

Experiment II (*Table II*)

Serum-virus neutralization. Serum samples, collected from ducks that had been treated with the virus were pooled and examined for the presence of neutralizing antibodies by the *in vitro* method. Tubes were put up containing serum and virus and, after two hours contact at room temperature (18°C.), or after 18 hours contact at 37°C., were inoculated into susceptible fowls and fertile 11 day-incubated hen eggs (only fowl inoculation has been shown in Table II). Suitable control tubes were also prepared using the serum of ducks that had not been inoculated with the virus. In no case was the virus neutralized, since the test fowls and chick embryos died of the infection.

TABLE II

Serum virus neutralization tests

Contact of serum-virus mixture for	Date	Fowl No.	Inoculation details (1·0 c.c. subcutaneous)	Result
2 hours room temperature	1-1-40	2080	Pooled duck serum obtained after virus inoculation + virus, equal parts	Died of Newcastle disease 8th day
Do	Do	2082	Do but with serum diluted 1 in 10	Do 7th day
Do	Do	2078	Normal duck serum + virus, equal parts	Do 5th day
18 hours 37°C	16-1-40	2109	Pooled duck serum obtained after virus inoculation + virus, equal parts	Do 8th day
Do	Do	2108	Normal duck serum + virus, equal parts	Do 6th day

CONCLUSIONS

The Pennine ducks and ducklings used in this work failed to react to small or big doses of virus or to contact infection. The serum from ducks inoculated with virus revealed no antibodies.

In confirmation of Dobson's [1939] findings, the ducks and ducklings used in this work were resistant to Newcastle disease virus.

SUMMARY

The ducks and ducklings used in this work were found to be resistant to Newcastle desease virus.

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ABSTRACTS

Female aspects of relative fertility in sheep. R. B. KELLY (1939). *Aust. vet. J.* **15**, 184

KNOWLEDGE concerning the fundamental principles of the physiology of reproduction owes much to the painstaking observations of laboratory animals such as rabbits and guinea-pigs, but relatively recently a few attempts have been made to obtain that information concerning the larger farm animals. It has been inferred commonly that what has been observed in small animals also occurs in other species. The anterior lobe of the pituitary gland activates the gonads alike in birds and man and therefore in broad principles the above inference is justified. Although in the rabbit ovulation is induced by coitus and even by mechanical irritation of a like nature it would be quite incorrect to infer that this is so for the ewe in which ovulation is quite independent of such a stimulus. In the ewe oestrus is the first phase of the reproductive function. Oestrus by itself is not a sign of fertility though it is a necessary preliminary to conception. There is little interbreed variation in the duration of dioestrus in domestic ewes. The normal period is 17 days. A range 16-18 is common, however 15 or 19 days interval must also be regarded as normal. The so-called 'silent heat' occurs most frequently in Merino ewes. In these cases oestrus usually reappears after a period which is a multiple of the normal. During silent heat no odoriferous substances attractive to the ram are produced. Recurrent intervals of sexual excitement alternating with a period of dioestrus constitute a breeding season. The long break between these seasons is known as the period of anoestrus or sexual inactivity.

According to Marshall, sexual periodicity is primarily a function of the gonads. In England most breeds of sheep have an extended breeding season during late summer, autumn and winter. In Scotland it is later. While the breeding season is more limited in countries further north than Scotland, a continual activity is approached within the tropics. Marshall also observed that Dorset Horned and Merino breeds of sheep are peculiar in that they come on heat throughout the year. The author's observations on three groups of Merino ewes and a group of Border Leicester × Merino cross-bred ewes showed a well-defined periodicity in the percentage of ewes coming into oestrus. In these four groups there was a decrease in the incidence of oestrus during the spring followed by an increase in the summer months and higher levels maintained during late summer, autumn and winter.

The importance of periodicity in sexual activity cannot be over-estimated in Australia where the statements of Marshall and others are accepted, since, if there is a definite periodicity as observed above then brief paddock matings will give unsatisfactory results. Again genetic factors play an important part in the periodicity. Some wild types and domestic breeds of sheep (the Scottish Blackface) are either monoestrus or nearly so; and others (Merinos) approximate a completely polyoestrus condition. The long wool breeds have a marked autumnal periodicity in sexual activity and this is accepted as due to hereditary make up. It is further suggested that the observed periodicity of sexual activity within the Australian Merinos is the result of out-crossing with long wool sheep during the foundation of the breed.

Marshall attributes the reproductive rhythm to three things (1) Metabolical (2) general environment and (3) exteroceptive stimuli. The observations of the author do not support the first two points of Marshall and other workers. It has been observed that light has a marked, if indirect, effect upon the gonads and that the essential stimulation is of the anterior lobe of the pituitary. The effect of daylight on sexual activity is discussed and according to the observations of the author, increased sexual activity in ewes was observed during decreasing hours of daylight. The fundamental phenomenon of reproduction is discussed showing its complexity and indicating that reduced fertility results from a number of causes. Fertility is defined as the ability of a male or female to beget or bear progeny. Fertility may be high or low and it is determined by the number of off-springs they produce during their breeding life. (The breeding life of a Merino ewe is divisible into three periods, youth, maturity and old age. The age factor on fertility indicates a range of 40 per cent between youth and early maturity (three years) and this is maintained up to the age of eight and then the level falls.) Normally fertile Merino ewes can experience 95 parturitions *per centum* of ewes adequately mated. Twinning is more characteristic of British breeds than of Merinos. British breeds have borne at least 70 per cent twins while for Merinos of all ages it was only 8 per cent. The breeding records of 89 ewes, each born in a twin pair, showed that only 20 of these bore twins and 69 did not. Some workers are of opinion that twinning is capable of being inherited as a recessive unit character. On the other hand the experience of the author and few others shows that the incidence of twinning is highest when the ewes are either naturally or artificially 'flushed' prior to or during mating and also that the age of the ewe is important. The ages when most twinning occurs were from 7-10 years and during this period the incidence was 33 per cent higher than the general average. Factors affecting low lamb markings and the methods to be adopted to prevent these are also discussed. [M. K. S.]

Sulphanilamide in animals : dosage and tolerence. A. W. STABLEFORTH and S. L. HIGNET (1942). *Vet. Rec.* **54**, 525

HORSES, cows and dogs were given varying doses of sulphanilamide either single or repeated *via* the subcutaneous or oral routes and estimations of the concentrations in blood and milk were made at varying intervals. Detailed observations of symptoms and any toxic effects exhibited by experimental animals were recorded.

Single doses given varied from 1 gm. per 5 lb. body weight to 1 gm. per 20 lb. weight, while in others continued dosage was maintained to give a concentration of about 10 milligrams per cent in blood and milk respectively. In cows serious toxic symptoms developed if the drug administration was continued over seven days. The most common symptoms of sulphanilamide overdosing in animals were lack of muscle coordination, anorexia and sleepiness. The drop in milk yield reached a miximum of 40 per cent in cows receiving an initial dose of 1 gm. per 5 lb. weight and maintenance doses totalling 1 gm. per 10 lb. daily for five to seven days.

From the results of many experiments carried out it was concluded that for therapeutic purposes it is desirable to maintain a concentration of about 10 mg. per cent. On this basis the doses recommended for different animals are,

dogs, 1 gm. per 15 lb. body-weight with the maintenance dose the same daily; for horses, 1 gm. per 10 lb. weight with maintenance dose from 1 gm. per 20 lb. to 1 gm. per 10 lb. wieght; and for cows, 1 gm. per 10 lb. weight with maintenance dose the same.

Some additional experiments were made on other species from which it is concluded that sheep may be treated like cows and pigs like horses. [J. A. I.]

The influence of dietary factors on egg-shell quality. I. Phosphorus, II. Calcium. R. J. EVANS, J. S. CARVER and A. W. BRANT (1944). *Poult. Sci.* 23, 9 and 36

I. ALTHOUGH a considerable amount of work has been published on the mineral metabolism of laying hens, there is little agreement in the literature regarding the phosphorus requirements. The authors have studied the phosphorus requirements of laying hens and determined the influence of different levels of phosphorus on egg-shell quality. Single comb White Leghorn pullets, kept in laying cages, were fed 11 different diets containing 1·5, 2·5 and 3·0 per cent calcium. The phosphorus levels of these diets were 0·6, 0·8, 1·0 or 1·2 per cent and the vitamin D content was 60 A.O. A.C. chick units per 100 gm. of diet. Birds receiving 0·8 per cent of phosphorus in the diet gave better results on the whole than those fed any other levels at a 2·5 per cent level of calcium during the first four months of the experiment. No significant differences in production, egg-shell weight, egg-shell thickness or shell smoothness were observed between 0·8, 1·0 and 1·2 per cent phosphorus used at 2·5 or 3·0 per cent level of calcium during the last six months.

II. The general procedure followed was the same as that used to determine the effect of the dietary phosphorus level on egg-shell quality. Calcium levels of 1·0, 1·5, 2·5, 3·0 and 3·5 per cent, and phosphorus levels of 0·6, 0·8 or 1·0 per cent were used. Hens receiving 3·0 per cent calcium in the diet gave more satisfactory results than those receiving higher or lower levels when egg-shell thickness was used as the criterion. Production and egg-shell quality were considerably decreased when the dietary calcium level was reduced to 1·0 per cent. A level of 2·5 per cent calcium gave as good production as one of 3·0 per cent but the latter level of calcium was found necessary for the production of thicker egg-shells. [S. B.]

Enzootic bovine haematuria (red-water of cattle) in British Columbia. J. C. BANKIER (1944). *Canad. J. comp. Med. vet. Sci.* 101, 146 and 178

THE disease is prevalent in regions widely separated and proximity has no relation to prevalence. The majority of 'red water' farms consisted of land partially cleared of timber, and deficient in pasturage. The soil was coarse, sandy and deficient in organic matter. The disease was found to be associated with certain farms rather than the type of soil. Manuring the land for years with lime and superphosphate did not prevent the disease, nor did the addition of mineral supplements to the diet. On a few farms top-dressing of the soil with gypsum together with crop rotation is said to have controlled the disease. Plant surveys failed to incriminate any particular regional flora incidental to the disease. Great variation was observed in the incidence in different herds maintained under identical conditions. The introduction of sick animals into clean areas neither resulted in spread of the disease nor retarded the clinical course.

The disease is characterized by the presence of a haematuria intermittent in the early stage and of short duration. The quantity of blood increased after each successive attack and coagulated blood voided. Sometimes blood clots caused retention of urine and death from uraemia. No systemic disturbance was noticed unless the loss of blood was heavy. Age, breed, sex and heredity had no bearing on the incidence of the disease: animals from 18 months to 13 years were equally affected. The lesions were confined to the inner surface of the urinary bladder, and varied from petechiation to small pedunculated vascular growths extending to the lumen of the bladder or to excessive granulations resembling papilloma. The vertex of the bladder was rarely involved. The coagulation time of the blood and the pH and sp. gr. of the urine were normal. The findings of Datta were not confirmed. Feeding experiments conducted on haematuria farms failed to incriminate water supply, pasture and feeds as causal factors, but when the animals were supplied with feeds from non-redwater areas the onset and the progress of the disease were considerably delayed. However, the feeding trials as well as field observations indicated that the disease was in some way associated with the soil content of red-water farms. No essential difference was noticed in the output of urinary inorganic sulphate in animals supplied with feeds from red-water and non-redwater farms. These results fail to confirm the Australian findings. Chemical analysis of soils from red-water and non-redwater areas gave no significant results as regards the content of Mn, Na, K, Ca, Mg, Al, Fe, Co and Se. No essential difference was noticed in Na, K, Ca, Fe, Si, P, Mg, Zn, Pb, B and Sr contents of the sera of healthy and sick animals. Experiments indicated that top-dressing of the soil with gypsum at 300 lb. per acre and feeding of small quantity of this salt as mineral supplement were beneficial to some extent as control measures. The results were, however, not conclusive. [N. S. S.]

The role of practising veterinarian in the control of coccidiosis. A. B. WICKWARE (1942). *Canad. J. comp. Med.* 6, 352

THE author in this article, having fully realized the importance of poultry husbandry to meet the exigencies of war, has indicated the part that can be played by the practising veterinarian in checking the sudden outbreaks of infectious diseases of poultry. It contains much useful information of immense value to both poultrymen and veterinarians. Briefly, differential diagnostic features of infections with six different species of *Eimeria* are as follows:

E. tenella infection—coecal coccidiosis. Chicks three to ten weeks of age are affected; blood is passed in the droppings; acute cases are marked by purplish red dilated coecal, pouches.

E. necatrix infection—upper and middle intestinal coccidiosis. Affects chicks ten weeks of age and above, occasionally younger birds; produces sudden deaths in apparently healthy flocks; head, appendages may be cyanosed; droppings are devoid of blood; blanching of the tissues occurs due to extensive haemorrhage into the small intestine.

E. acervulina infection—upper intestinal coccidiosis. Nature of infections chronic; in acute cases entire mucuous surface appear grayish, mottled and thick; droppings devoid of blood.

E. maxima, *E. mitis* and *E. praecox* infections cause little damage to the mucosa.

Sporulation time of different fowl coccidia varies from 21 to 48 hours, and therefore to control outbreaks the life-cycle should be interrupted at this stage. With the onset of listlessness and inappetance in the growing stock, especially in the absence of respiratory symptoms, coccidiosis should be suspected and the entire flock shut up in quarters from which droppings and litter could be removed every 24 hours for at least a period of one week. A dose of epsom salt (1 lb. for each unit of 150 adult birds) at this stage—either in mash or drinking water—helps to purge out many harmful forms of Coccidia. Withholding of whole grain is advised during

this period. At the end of the confinement period the flock should be placed on a new range with an access to liberal quantity of fresh green feed. Recovered adult fowls become reservoir of infection for the young birds.

Cross-infection from wild birds to chick does not occur. The process of immunization against coccidiosis by exposing the birds to a moderate amount of infection in early life has much to commend it to the experienced poultrymen, but to the average breeder the measure of separate brooding and rearing is the only safeguard. In case of annual recurrence of the disease it is advisable to market the adult birds in the spring and purchase new eggs or chicks from incubator instead. [H. N. R.]

Studies on the survival of Johne's bacilli. R. LOVELL, M. LEVI, and J. FRANCIS (1944). *J. comp. Path.* 54, 120-9

JOHNE'S bacilli are believed to survive for prolonged periods outside the animal body, but this opinion is based largely on the similarity of the organism to the tubercle bacillus and not on any direct experimental evidence. The authors carried out a series of experiments designed to test the viability of Johne's bacillus under a variety of conditions. These experiments may be considered under three headings:

(A) SURVIVAL OF JOHNE'S BACILLI IN STERILIZED WATER AT ROOM TEMPERATURE

The viability of Johne's bacilli was tested in sterilized distilled water, tap water and pond water containing dried mud. Cultures of Johne's bacilli were mixed in varying amounts with each kind of water and the mixtures stored in bottles in a cupboard at room temperature. At approximately monthly intervals, 10 c.c. samples were withdrawn from each bottle, centrifuged and the deposit sown on Dunkin's medium without dye. Duplicate cultures were made after treating the deposit from an equal quantity with 10 c.c. of 10 per cent antiformin for 15 minutes. Johne's bacilli were recovered from each of the three waters after nine months, but not after 13 months. The *p*H of the waters varied during the course of the experiment between 6·4 and 6·8 for the distilled water, 7·1 and 8·0 for the tap water and 5·3 and 5·9 for the pond water. The temperature fluctuated between 49° and 79° F.

(B) SURVIVAL OF JOHNE'S BACILLI IN NATURALLY INFECTED INTESTINAL SCRAPINGS IN UNSTERILIZED RIVER WATER IN THE OPEN AIR

Intestinal scrapings from an infected case of disease were finely emulsified and mixed with river water collected from a flooded meadow. The mixture was stored in a bottle in the shade in a suitable part of the building, and samples were withdrawn at monthly intervals and cultured after treatment with antiformin. Johne's bacilli were recovered from the water after 113 days' exposure. During the early part of this experiment, the weather was very severe and between the first and the 30th day the water was actually frozen.

In a subsidiary experiment, in which a culture of Johne's bacilli was substituted for the intestinal scrapings, the bacilli were recovered only after the 1st day but not after the 30th day. The authors are unable to give any explanation of this anomalous result.

In another experiment, river water to which had been added an emulsion of infected intestinal scrapings was placed in two shallow earthenware bowls, 24 in. × 4 in. one of which was kept in the shade and the other exposed to sunshine. Johne's bacilli were recovered from the bowl kept in the shade after 135 days but not after 163 days. From the bowl kept in the sunny area, bacilli were isolated after 163 days and not after 218 days. During the period of the experiment, the atmospheric temperatures varied between 20° and 64° F. and during the very cold weather the contents of the bowl kept in the open were often frozen; they were never dry and the actual amount of the mixture varied with rainfall.

(C) SURVIVAL OF JOHNE'S BACILLI IN NATURALLY INFECTED FAECES EXPOSED TO ATMOSPHERIC CONDITIONS

Fluid faeces were collected from a natural case of disease and placed in a shallow porous bowl similar in size to those already described. This was placed in the open air exposed to the full effect of sunshine and rain. The contents of the bowl were sometimes fluid, sometimes frozen and at times dried by the sun. The temperature varied between 26° and 74° F. Johne's bacilli were recovered for periods up to 246 days.

In another experiment, infected faeces were exposed to air, a portion in a porous bowl and some in an enamel bowl. The temperature fluctuated between 27° and 74° F. and the contents of the porous bowl became dry towards the latter part of the experiment. The contents of the enamel bowl were, however, prevented from drying by the addition of fresh water on one occasion. Under these conditions Johne's bacilli were found to be viable in the porous bowl after 152 days and in the enamel bowl after 208 days.

A number of subsidiary experiments carried out with fresh samples of faeces yielded results more or less similar to those above described.

In all the experiments, there was a progressive diminution in the number of bacilli with the result that the number of colonies obtained in culture towards the end was always less than that at the beginning.

The viability of Johne's bacilli depends upon a number of factors, such as the intensity of infection, temperature, moisture content of the material, etc.; of these the moisture content appears to be the most important.

Summing up their results, the authors conclude that they afford ample confirmation of the generally held view regarding the longevity of Johne's bacillus and lend strong support to the measures often advocated for the control of the disease, such as the breaking up of dung and manure, leaving infected pastures ungrazed for over a year or more, draining or fencing of ponds, etc.

[It may be pointed out that these results were obtained in England under the conditions obtaining in that country and they should not be interpreted too rigidly in their application to this country where the climatic conditions are different]. [L. S.]

Yeast as a protein supplement for pigs and its relation to the appearance of rickets. R. BRAUDE, S. K. KON and E. G. WHITE (1943). *J. comp. Path.* 53, 161

THE authors in this article record the results of feeding experiments with yeast as a protein supplement for fattening pigs in peace-time. From a practical stand-point, the results support the conclusions of many workers regarding the high value of yeast as a protein supplement for pigs, but as in the present experiments where the object was to ascertain whether yeast could wholly replace the protein supplement, some difficulties resulting from feeding large quantities of yeast were observed.

Three series of experiments have been conducted and in these the effects of yeast at the levels of 8 and 20 per cent in the diet have been observed. A diet containing 8 per cent of yeast, with no mineral supplements to make up a balanced ration, was found to produce adverse symptoms in some of the experimental animals, whereas an addition of extra calcium supplement in the form of calcium carbonate (Ca con-

tent 38·5 per cent) prevented their appearance. A 20 per cent yeast diet even when supplemented with limestone or calcium carbonate to the extent of 1·8 to 2·5 per cent was found to be harmful, whereas the addition of cod-liver oil (declining from 1 per cent to 0·3 per cent) prevented the occurrence of symptoms.

The main symptoms in the experimental animals were inappetance, abnormality in gait, appearance of tetanic spasms and the suppression of urination and defaecation. The nature of the trouble was considered to be rickets on the basis of post-mortem findings, and these comprised a puckering of the articular cartilages of the limb bones (which could explain abnormal gait), marked thickening of the periarticular connective tissue and abnormal width of the epiphyseal cartilages. Histologically, there were fibrosis of the metaphyseal marrow and some rarefaction of the shaft of the bone. Throughout the sections, there was observed an excess of osteoid tissue, both as pure osteoid trabeculae and as wide margins to the bony trabeculae. Pronounced osteoporosis was also in evidence.

The paper contains an excellent review of literature dealing with rickets in pigs in general, and in relation to yeast feeding, a passing reference has been made to ill effects arising from its use in pigs. In discussing the possible role of yeast in bringing about the condition, the authors have laid special emphasis on the high P content of this supplement which results in a marked shift in the ratio of calcium to available phosphorus. [P.G. P.]

Storage changes in spray-dried whole egg powder. L. S. STUART, H. H. HALL and EDNA E. DICKS (1942). *U. S. Egg and Poul. Mag.* **48,** 629

BACTERIAL and chemical analyses were made of samples of spray-dried whole egg powder immediately upon receipt from the driers. They were then stored at 30°C. at relative humidities varying from 20 to 100 per cent for a period of 60 days, the analyses repeated, and the changes undergone during storage studied.

The results of these experiments indicated that spray-dried whole egg powder which had been properly dried would not mould if stored at relative humidities of 85 per cent or less at 30°C. or below, as well as that bacterial growth would not occur in stored samples of similar material unless the relative humidity of the storage atmosphere exceeded 90 per cent at 30°C. Changes in the solubility of the egg powder during storage were found to be much more pronounced when the moisture content of the powder exceeded 5·0 per cent. Bacteria of the coliform group, if present, tended to remain viable during storage. Changes in the total bacterial count, as measured by the dilution plate method, were influenced by the amount of moisture initially present in the sample. When the moisture concentration was 5 per cent or less bacterial death appeared to be greater as the moisture content was reduced. With moisture concentrations greater than 5·0 per cent, the reverse seemed to be true, i.e. until it became high enough to allow of bacterial growth. Death of bacteria in powdered dried egg at moisture concentrations higher than 5·0 per cent seemed to be associated with the chemical changes involved in the loss of solubility. It was observed that marked decreases in the solubility of the stored material might occur independently of the changes in the free fat acid content, formol nitrogen value or total free carboxyl groups. That pronounced increases in the content of these latter chemical constituents might be brought about as a result of bacterial or mould growth during storage at high relative humidities was also noticed. Changes in the pH values of stored dried eggs became more pronounced as the moisture content of the samples increased from 1·94 per cent to 9·96 per cent. The fact that solubility changes, decreases in the total bacterial plate count and decreases in pH values were influenced by the moisture content of the stored spray-dried whole egg powder indicated that the most important single factor in the preservation of good quality during storage may be control of the moisture content of the product. This would then involve the initial production of dried egg powder containing not more than 5 per cent moisture and adequate care in the subsequent storage and handling processes to prevent moisture absorption.

The above experiments are only of a preliminary nature, and the conclusions are consequently only tentative. There is, however, urgent need for more specific information on such practical points as the most favourable relative humidity for storage as well as the actual nature of the chemical changes taking place in stored dried whole egg. [T. S. K.]

Rotenone in low concentration as a tickicide and insecticide for house pets. ZACARIAS DE JESUS and ROFINO B. GAPUZ (1940). *Philipp. J. Anim. Industr.* **7,** 391

IN view of very close association of the dog and the cat as house pets with the members of the household, tickicides and insecticides for these animals should be neither toxic nor repulsive. They should not be in the form of emulsions as they will soil the clothes and furniture. A mixture of rotenone and cassava starch in concentration of one and two per cent rotenone was found effective as a tickicide for dogs. Twenty to 50 grams of the preparation were necessary to treat one dog. *Rhipicephalus sanguineus* and *Dermacentor* sp. were killed in from 24 to 72 hours and these ticks remained attached to the skin of the host until they died and dried up. The mixture has been reported by the authors to have great insecticidal values against cat and dog fleas. The fleas die soon after the application. When applied liberally all over the body, head and appendages of the infested dogs and cats, one application was found sufficient to effect complete destruction of the ticks and fleas. In the case of gravid ticks, it was found necessary to repeat the treatment the following day or on the third day due to the high resistance of the ticks to the tickicide at this stage. Trials made on guinea-pigs had shown that the mixture was also effective against lice, killing them in a short time. To prevent the rotenone from deteriorating, the mixture should be kept in a well-stoppered bottle and should not be exposed to direct sunlight. [B. C. B.]

Feeding urea to dairy cows. J. G. ARCHIBALD (1943). *Massachusetts Experiment Station Bulletin* **406,** 16

IN three years' trials with dairy cows with double reversal and continuous rations, it was found possible to replace as much as 42 per cent of the total N in the grain and 25 per cent of the total N in the entire ration by urea. In making the substitutions, urea replaced standard protein concentrates such as cottonseed meal, soybean meal and corn gluten feed. Urea gave some favourable results, but it was not quite on a par with the standard protein concentrates for maintenance of milk production.

Some factors influencing reproductive efficiency of range cattle under artificial and natural breeding conditions. J. F. LASLEY and R. BOGART (1943). *Missouri Station Research Bulletin* **376,** 56

ARTIFICIAL insemination proved a practical and efficient method for breeding nearly 2,000 range cows over a period of three years. Of the cows inseminated one or more times, 78·8 per cent produced calves and required an average o

1·63 inseminations per calf. Study of the concentration, volume and motility of the semen from 12 range bulls showed much variability, but in general, fertility was related to semen volume, concentration of sperm, number of sperm per ejaculate, percentage of live sperm and percentage of sperm resistant to ten minutes' exposure to 0°C. in egg yolk-buffer. Through the use of stored semen and dilution, the number of cows bred with a single ejaculate was greatly increased. Uterine insemination gave the best results with about 200 cows, as contrasted with groups of 20 cows each inseminated by the cervical and vaginal methods. Fertility below the average was obtained when less than 800,000,000 total sperm, or 275,000,000 live sperm or 175,000,000 sperm resistant to cold were employed for fertilization purposes.

The use of dried whey and blood meal in the raising of calves on limited amounts of milk. I. L. HATHAWAY, G. W. TRIMBERGER and H. P. DAVIS (1943). *Nebraska Experiment Station Research Bulletin* **132**, 19

DRIED whey and blood meal were successfully utilized to replace various portions of skim milk in calf rations from three weeks to six months of age. The study was based on the weekly weights and measurements of six lots of seven to nine calves each with variance analyses of the results. These were fed 50, 100, 150, 200, 250, or 300 lb. (23, 45, 68, 91, 113, or 136 kg.) of skim milk in the different lots, with replacements of 50 lb. (23 kg.) of skim milk by 6·8 lb. (3·1 kg) of a mixture of dried whey and blood meal 3·2 parts to 1. Other feeds included in the ration were alfalfa hay, a grain mixture and a vitamin concentrate. The calves in five of the lots made average daily gains of 1·5 lb. (680 m). The supplement fed at the rate of 6·8 lb. (3·1 kg.) proved a satisfactory substitute for 50 lb. (23 kg.) of skim milk in feeding healthy dairy calves approximately three weeks of age and weighing not less than 104 lb. (47 kg.). These conclusions were based on variance analyses of the weights obtained.

The feeding value of clover-molasses silage for milking cows. A. D. PRATT and C. W. HOLDAWAY (1943). *Virginia Experiment Station Bulletin* **353**, 15

MOLASSES at the rate of 2 and 44 per cent added to clover silage in the ensilage cutter produced a silage equal or superior, on a dry-matter basis for milk production, to clover hay. The studies were conducted in three experiments with three cows each fed by the reversal method, with clover silage and clover hay or clover silage alone as the sole roughage with a grain ration. Corn silage was fed for one year.

MGIPC—M—III-1-105—5-8-46—520.

ORIGINAL ARTICLES

BACTERIOLOGY OF BOVINE MASTITIS IN INDIA WITH SPECIAL REFERENCE TO THE INCIDENCE OF *STREPTOCOCCUS AGALACTIAE*

By Ram Narain Mohan, Imperial Veterinary Research Institute, Izatnagar

(Received for publication on 26 April 1945)

Veterinary clinicians in India have been quite familiar with mastitis as an affection of milch cattle, but it was not until the beginning of the last decade that the subject received serious consideration. The investigations of various workers in Europe and North America supplied the much needed stimulus in this direction, so that from 1935 the subject has been given more attention than before. In this paper is given a consolidated summary of the work done from 1935 to 1942. This includes a systematic survey of the incidence of *Streptococcus agalactiae* infection in some dairy herds. The main object has been to obtain some preliminary knowledge of the bacteriology of bovine mastitis in India. The work was started primarily to determine the common bacterial types associated with it, and secondly, the extent to which *Str. agalactiae*, the most important and common cause of chronic streptococcus mastitis in other countries, is responsible for udder infections in this country.

The observations made are described under two sections I and II.

Section I

Under this head is described the preliminary work done from 1935 to 1940 to determine the types of bacteria associated with mastitis, and especially to see if *Str. agalactiae* could be commonly isolated.

Material and methods

The material examined came, as a rule, from cows and buffaloes affected with clinical mastitis in the experimental dairy herds at Mukteswar or from different parts of the country. In the case of the Mukteswar cows, the material consisted of mixed udder milk or more commonly milk from affected quarters. The technique adopted was to streak the centrifuge deposit on blood or plain-agar slants for primary isolation of bacterial types. In many cases, cultures were obtained with a fair degree of purity. The material received from the field was generally milk or a more or less purulent udder secretion, swabs of milk or pus, and in one case a portion of the affected mammary gland preserved in glycerine. The cultural technique adopted with these specimens was similar to that used for milk from the Mukteswar cows. In many cases films of the udder secretion or milk deposit were also examined for bacteria and pus-cells. Many of the Mukteswar cows and a couple of 'field' cows were reexamined.

The bacteria isolated in pure culture and suspected of being connected with the disease were studied. In the early stages of the work, the system of classification recommended by Bergey was used. This largely explains why *Str. agalactiae*, though probably present, was not definitely identified until 1939, after which it became the general practice to submit all the streptococcus strains to the biochemical tests now recommended for classifying mastitis streptococci.

Results

Only the more important features are given below.

1. *Streptococci*. About 55 strains of streptococci were recovered from 45 animals (18 Mukteswar and 27 'field' animals). Few of these strains were subjected to a thorough examination, so that the identifications recorded would not satisfy modern standards. The more definite and reliable specific labels are considered below.

(*a*) *Str. agalactiae*. *Str. agalactiae* was isolated from ten cows and one buffalo; seven of these were 'field' cows. This number also includes one *Str. mitior* strain which on later examination proved to be *Str. agalactiae*. At least one other strain, which had been considered to belong to 'Mastitis Streptococcus, Group I' (Minett, Stableforth and Edwards) may have been a true *Str. agalactiae*, but it was not available for further examination.

(*b*) *Str. mitior*. *Str. mitior* was isolated from three cows, two of which were 'field' cases, and one buffalo. Perhaps all, and certainly one, of these strains should be regarded as *Str. agalactiae*. The buffalo strain was not available for further examination, but the animal itself was two years later found to be infected with *Str. agalactiae*.

(*c*) *Str. uberis*. *Str. uberis* was said to have been isolated from two 'field' cows, but the diagnosis was based on incomplete study. Organisms labelled 'haemolytic diphtheroids', probably *Corynebacterium pyogenes*, were also recovered from these cows.

(*d*) *Str. subacidus*. The characters of this organism are fully described in Section II. Strains of streptococci possessing such characters were recovered repeatedly from two Mukteswar cows. During the later survey work, one of these proved

to be infected with *Str. agalactiae*, but from the other *Str. subacidus* was again recovered.

(*e*) *Str. pyogenes*. *Str. pyogenes* (i.e. strongly haemolytic streptococci belonging to Lancefield's group A) was not isolated from a single case.

2. *Staphylococci*. *Staphylococci* considered to have pathogenic importance were isolated from about 20 animals. None of these strains was actually tested for its pathogenicity, but a few were found to be haemolytic and co-agulase positive. Their characters are further described in Section II.

3. *Diphtheroids*. Many of those isolated were obviously non-pathogenic. *C. pyogenes* was isolated in practically pure culture from purulent udder secretions of five 'field' cows and one 'field' buffalo. Two more strains from 'field' cows and labelled 'haemolytic diphtheroids' were probably true *C. pyogenes*. In all these, there was a history of a suppurative mastitis. The cultural and other characters of these strains corresponded to those of classical *C. pyogenes* from mastitis and other disease conditions, viz. small Gram-positive organisms, forming minute haemolytic zones in blood agar, digesting milk and gelatine and solid serum, and giving acid in lactose and not mannitol. Another character, perhaps not previously described, was their power of hydrolysing hippurate, like *Str. agalactiae*, but to a somewhat lesser extent, especially when grown in media containing a little ox-blood serum. This property was manifested by all the mastitis strains as well as by those from other sources.

4. *Bacterium coli*. *Bact. coli* and coliform organisms were isolated from several cows. From one cow and one buffalo at Mukteswar, haemolytic strains of *Bact. coli* were recovered repeatedly and this organism probably caused the somewhat acute mastitis occurring in these animals.

5. *Mycobact. tuberculosis*. Although a number of tests were made, there is but one case in which tuberculosis of the mammary gland was established by cultural and biological tests.

6. *Miscellaneous bacteria*. Among other bacteria of doubtful pathogenicity, the outstanding organisms were strains of *Proteus* and *Pseudomonas*.

Section II. Systematic survey

After *Str. agalactiae* as a cause of bovine mastitis in India had been definitely established, it was decided to institute a general survey of the incidence of udder infections due to this organism in a few organized dairy herds. The results are shown in Table I.

Material and methods

A total of 541 cows, including four buffaloes, belonging to 15 dairy herds in different parts of the northern India, were examined. The 16 cows included as Herd 13 really consisted of animals admitted to the Punjab Veterinary College from various parts of Lahore; they are herded in this way for descriptive purposes. Milk samples were taken from the individual quarters of nearly all cows available in the different herds. In a few cases, one or more teats were 'blind', and in each of two animals only one quarter was available.

As the main object was to determine the incidence of *Str. agalactiae* infections and since it was also desired to make some simultaneous observations on the incidence of *Staph. pyogenes* infections, the cultural technique employed was essentially the same as that used by the English workers [Minett, 1934], viz. to centrifuge about 10 c.c. of foremilk collected cleanly from individual quarters and to sow the deposit in 5 per cent ox-blood agar plates. As a rule, two plates were made from each sample. In the first half of the deposit and in the second one-tenth of this amount was taken. When the deposits were particularly heavy or when there was reason to suspect a high bacterial count, plates were sown from further dilutions. Plates were examined at 24 and 48 hours incubation and the type of colonies noted. Suspicious colonies were transferred to one per cent serum broth for morphological study and purification (blood agar surface). *Streptococci* were tested in the following manner. Type of haemolysis was noted in the primary growths from the deposits. Intensity and character of growth in serum broth were noted after 24 and 48 hours and morphology was studied (chain formation, Gram-staining). Fermentation tests were made in lactose, sucrose, mannitol, salicin, sorbitol, trehalose, raffinose and inulin, all in one per cent solutions and containing one per cent each of ox-serum and Andrade indicator. Also noted were final pH in glucose broth (one per cent), hydrolysis of sodium hippurate, changes in litmus-milk and methylene blue-milk (1:20,000). Unless otherwise stated, observations were made during one week at 37°C.

Results

The main results are summarized below. Table I should be seen again.

1. *Streptococci*. One hundred and twenty-four strains of streptococci, isolated from 87 cows and one buffalo, belonging to 12 herds, were classified as follows.

(*a*) *Str. agalactiae* was definitely diagnosed in two herds and suspicious strains were isolated from three more herds. The ten suspicious strains came from eight cows as follows: four from two cows at Izatnagar and six from six cows in the neighbourhood of Lahore. The ten authentic strains of *Str. agalactiae* were isolated from eight cows and one buffalo. Thus, in this survey the percentage of animals definitely infected with this organism is less than two. One of the infected herds at Mukteswar (Herd 1) contained at the time of

TABLE I

Incidence of streptococci, etc. from milk samples of 15 herds

Herd No.	Pla	Time of examination	No. of cows examined	Total streptococcus strain isolated	Str. agalactiae		Other mastitis streptococci	Streptococci, probably non-pathogenic	Staph. pyogenes strains
					Authentic	Suspected			
1	Mukteswar	June-July 1940	30 and 4 buffaloes	12 (from 10 cows and one buffalo)	4 (from 3 cows and one buffalo)		(i) *Str. uberis*, 2 (from two cows) (ii) *Str. dysgalactiae*, One suspicious strain	2 (from two cows) including one of *str. acidominimus*	30 (from 14 cows and 2 buffaloes)
2	do.	July 1940	12	24 (from 9 cows)				*Str. acidominimus* 24 (from 9 cows)	20 (from 7 cows)
3	Patna	April-May 1941	83	23 (from 18 cows)	6 (from 5 cows)			17 (from 13 cows)	40 (from 23 cows)
4	Izatnagar	December 1941	21	4 (from 2 cows)		4 (from 2 cows)			13 (from 6 cows)
5	Lahore	January 1942	99	3 (from 3 cows)				3 (from 3 cows)	16 (from 13 cows)
6	do.	do.	6						4 (from 3 cows)
7	do.	February, 1942	34						(from 3 cows)
8	do.	do.	21	1				1	9 (from 7 cows)
9	do.	do.	21	4 (from 4 cows)		2 (from 2 cows)		2 (from 2 cows)	(from 7 cows)
10	do.	do.	21						(from 3 cows)
11	do.	do.	21	2 (from 2 cows)				2 (from 2 cows)	9 (from 6 cows)
12	do.	March, 1942	30	1				1	(from 1 cow)
13	do.	do.	16	13 (from 11 cows)		4 (from 4 cows)		9 (from 7 cows)	(from 1 cow)
14	New Delhi	May, 1942	54	29 (from 20 cows)				29 (from 20 cows)	40 (from 25 cows)
15	Karnal	do.	68	8 (from 6 cows)				8 (from 6 cows)	8 (from 7 cows)
	Total	. . .	537 cows and 4 buffaloes	124 strains	10 strains	10 strains			

amination 30 cows, mostly crossbreds, and four buffaloes in milk. *Str. agalactiae* was isolated from three cows and one buffalo. There were also one cow and one buffalo in this herd previously shown to be infected with *Str. agalactiae.* Two other cows, which were apparently free from pathogenic streptococci in this survey, were found to show *Str. agalactiae* at a previous examination (Section I), but the quarters from which these organisms were originally obtained were 'blind' at the time of the survey. Obviously pathogenic streptococci were also recovered from five more cows of the same herd. It is interesting to note that in herd 2, Mukteswar, which consisted of the small Afghan and Kumaoni hill cows, *Str. agalactiae* was not encountered. From the second infected herd (Herd 3, Patna), six strains of *Str. agalactiae* were recovered from five of 83 cows examined. Seventeen more strains of streptococci, which could not be properly classified but which were probably of little pathogenic significance, were recovered from 13 other cows of this herd.

The main cultural and biochemical characters used for classifying *Str. agalactiae* and possessed by all strains accepted as *Str. agalactiae* in this work were as follows.

Haemolysis. *Str. agalactiae* was easily recognized by the comparatively small width and weak character of the β-haemolytic zones about the deep colonies in ox-blood agar plates after 24 hours incubation.

Growth. Growth in one or 5 per cent serum broth was moderately abundant, with clear supernatant and floccular sediment, sometimes faintly pigmented. The sediment was composed of medium to long chains of Gram-positive cocci.

Hydrolysis of sodium hippurate. This reagent was unmistakably hydrolysed (usual ferric chloride test, with unsown hippurate broth controls).

Final pH in one per cent glucose broth. This was generally low, towards 4·2.

Litmus-milk. Fair inocula produced rapid acidity with firm clot formation within 24 hours, with some decolourization at the bottom. On further incubation, a clear reddish whey was expressed.

Methylene blue-milk. Generally, the results varied with the size of the inoculum. Small inocula failed to grow. Heavy inocula caused a complete reduction and clot formation within 24 hours, followed later by progressive oxidation from the top.

Sugar reactions. All strains produced acid in lactose, sucrose, salicin and trehalose, but not in mannitol, sorbitol, raffinose and inulin.

(*b*) *Str. uberis.* *Str. uberis* (Mastitis Streptococcus, Group III, of Minett *et al.*) was isolated from two cows at Mukteswar. Both were α-haemolytic, gave abundant floccular growth in serum broth with clear supernatant, formed short to medium chains, hydrolysed sodium hippurate, produced a final *p*H 4·6-4·9 in glucose broth, acid in litmus-milk with small inocula but acid and soft clot within 24 hours with larger inocula, acid in lactose, sucrose, mannitol, salicin, sorbitol, trehalose and inulin, but not in reffinose.

(*c*) *Str. dysgalactiae. Str. dysgalactiae* (Mastitis Streptococcus, Group II, of Minett *et al.*) was suspected in one cow at Mukteswar, but the reactions were not quite typical. Except for the type of haemolysis, these characters were not very different from those manifested by strains labelled as *Str. subacidus.* The organism was α-haemolytic, grew abundantly in serum broth as a floccular pigmented sediment with clear supernatant, short to medium chains, a final *p*H of 5·4 in glucose broth, no hydrolysis of sodium hippurate, acid in sucrose and trehalose. Small inocula in litmus-milk and methylene blue-milk gave no change, larger inocula caused a slight and incomplete reduction later returning to normal.

(*d*) *Str. subacidus.* This was the label given to a number of strongly β-haemolytic streptococcus strains isolated from various cows at Mukteswar and elsewhere, sometimes repeatedly and in large numbers, and often in association with *Staph. pyogenes.* These streptococci were considered to be definitely pathogenic and their main cultural and biochemical characters were as follows: typical streptococcus colonies, both deep and superficial ones being surrounded by wide zones of clear β-haemolysis; abundant and sometimes pigmented floccular sediment and clear supernatant in serum broth, chains of medium length; final *p*H of 5·2—5·6 in glucose broth; hippurate not hydrolysed; acid and slowly-formed incomplete clot in litmus-milk with slight reduction at bottom; small inocula producing no change in methylene blue-milk but larger inocula causing complete reduction with clot formation; acid only in lactose, sucrose, and trehalose.

Two of these strains were tested for pathogenicity, using 0·5 c.c. serum broth culture intraperitoneally. Both were non-pathogenic at this dose.

(*e*) *Str. acidominimus.* Twenty-five strains, isolated from ten cows at Mukteswar, had the characters of this species. There was no evidence of mastitis in these cows and the streptococci appeared to be saprophytic. Their salient characters were: alpha-haemolysis; slight uniform turbidity in serum broth, short to medium chains; final *p*H 6·6 in glucose broth; comparatively weak but definite hydrolysis of sodium hippurate; no change in litmus-milk or methylene blue milk; very weak acid in lactose, surcrose and trehalose.

(*f*) *Unclassified.* Seventy-three strains isolated from 56 cows could not be properly classified. Most were considered of no direct pathogenic significance.

2. *Staphylococci.* These commonly found organisms were divisible into two main groups [Minett, 1937].

(*a*) *Saprophytic staphylococci.* (i) Colonies unpigmented or white pigmented, superficial ones often resting on a bed of clear haemolysis but deep colonies either non-haemolytic or surrounded by zones of greenish discolouration; (ii) Colonies pigmented yellow or somewhat like '*Staph. aureus*' or, as in one case only, like '*Staph. citreus*', generally with no evidence of haemolysis around either deep or superficial ones; (iii) Small colonies, the deeper ones more minute and often surrounded by fair-sized zones of intense greenish discolouration. Organisms of the last group had a characteristic tendency to form tetrads. In ox-blood agar plates, their minute deep colonies were sometimes liable to be mistaken for streptococcus colonies. The few strains studied of these different types were found to be non-proteolytic and failed to form coagulase or soluble haemolysis.

(*b*) *Staph. pyogenes.* Colonies generally pigmented like '*Staph. aureus*'. Deep colonies were surrounded (i) in several cases, by wide and well-defined zones of darkening, characteristic of β-toxin; (ii) in a few cases, by moderate-sized zones of clear haemolysis,

characteristic of α-toxin; and (iii) in most cases, immediately by comparatively narrow zones of clear haemolysis and peripherally by wider zones of darkening, indicative of both α-and β-toxin. Storage in the cold brought about progressive changes in these different types of haemolysis characteristic of pathogenic staphylococci of bovine origin. Superficial colonies were generally haemolytic. A few strains studied were found to produce soluble haemolysin; they were actively proteolytic (gelatine), generally fermented mannitol and invariably formed coagulase for rabbit plasma.

Staph. pyogenes was encountered in 204 quarters of 126 cows and two buffaloes, i.e. in nearly 24 per cent of all animals examined. No herd was found completely free from infection with this organism and in some the incidence was nearly 60 per cent. In many cases the colonies obtained were very numerous, and it seems that *Staph. pyogenes* may be regarded as the commonest cause of udder infections in this country. It was, at any rate, found to be common in milk from cows which had developed acute inflammation of the udder, generally *post-partum*. With suitable treatment and care, such udders tended to return to normal, but the worst-affected quarters continued to excrete the organism in gradually decreasing numbers for some time. Similar affections also seem to be associated with other bacteria and occasionally, especially when examined quite early in the onset, it may not be possible to recover any organism of pathogenic significance.

Discussion

It is hoped that this work may provide preliminary information on the general bacteriology of the diseased bovine udder in India and may furnish some information on the incidence of different types of streptococci, staphylococci, and other organisms recognized to be commonly associated with the disease in other countries.

The methods used for streptococci were restricted to their cultural and biochemical characters. Serological typing was not undertaken, partly because objects of the work did not demand it, partly because the typical strains of *Str. agalactiae* can be recognized without much difficulty by the methods used and partly because facilities for serological work were not available.

Since systematic examination for udder induration, etc. was not done, it is not possible to give accurate information about the correlation between the different bacteria and the various kinds of udder affections. However, some information of a general character, based partly on the clinical history supplied and partly on a few clinical examinations has been incorporated.

Summary

This paper gives an account of preliminary investigations, carried out from 1935 to 1942, on the general bacteriology of mastitis in cows and buffaloes in India. Systematic examination of the udder for indurations, etc. was not taken up.

The first section deals with the examination of over 100 specimens of udder secretion from 85 cows, including a few buffaloes, affected with clinical mastitis in different parts of the country. The second section is concerned with a systematic examination of 541 cows from about 15 different dairy herds in the northern India.

3. Bacterial infections of the bovine udder in India correspond fairly, in type and cause, to similar infections in other countries.

The existence of *Str. agalactiae* has been established in both cows and buffaloes at a number of places, but the incidence is low. In the preliminary work, several strains of *Str. agalactiae* were recovered from cows and buffaloes affected with clinical mastitis. In the systematic survey, ten authentic strains and ten suspicious strains of *Str. agalactiae* were isolated from cows and buffaloes belonging to five herds.

Other streptococcus groups occasionally found were *Str. dysgalactiae* in one cow with subacute mastitis, *Str. uberis* in two cows with a history of mastitis, and *Str. subacidus* from a few cows mostly with a more or less acute mastitis. There were also several strains of streptococci, mainly from apparently healthy quarters, which were not properly classified. There was no authentic case of *Str. pyogenes* (Lancefield group A) infection.

Among other well-recognized pathogens of the bovine udder were:

(*a*) *Staph. pyogenes*, of which some 250 strains were isolated from nearly 140 cows; this organism was held responsible, alone or in conjunction with others, for some acute *post-partum* mastitis and for much chronic low grade mastitis.

(*b*) *Corynebact. pyogenes*, was isolated, often in pure culture, from about five cows and one buffalo with suppurative mastitis.

(*c*) *Bact. coli*, and other coliforms, were isolated from several animals and seemed to be definitely pathogenic in at least one cow and one buffalo.

(*d*) Other organisms of possible importance were three strains of *Proteus* and one strain of *Pseudomonas*.

Acknowledgements

Space does not permit individual acknowledgement to all officers who were on different occasions connected with this work. The two outstanding contributions have been from Messrs P.R.K. Iyer and B. N. S. Chowdhury. The former was responsible

for many of the findings reported in Section I, and the latter did much of the work on animals at Lahore. The writer is grateful to Mr J. F. Shirlaw for encouragement and help in arranging facilities. The systematic survey reported in Section II was initiated by Dr F. C. Minett, to whom I am also indebted for arranging and correcting this paper.

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SOME GENITAL ABNORMALITIES OF THE INDIAN COW AND BUFFALO, WITH REFERENCE TO ANATOMICAL DIFFERENCES IN THEIR REPRODUCTIVE ORGANS

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(With Plates VI-XI)

This work was commenced as part of a general investigation of sterility of cows and buffaloes, but it soon became clear that the present knowledge of the buffalo's genitalia is very slight indeed. Consequently, a short immediate contribution towards a better knowledge of this subject was deemed desirable.

I. Comparative genital anatomy

Uterus. Plate VI depicts the uterus and adjacent organs of the cow and buffalo. As the average Punjab buffalo (Murrah type) is larger than the Punjab cow (Sahiwal type), so is the former's uterus generally larger than the latter's. Table I gives some comparative mean measurement of these organs. The organs also differ considerably in their colour and texture. The uterus of the zebu is cream-coloured and faintly tinged with pink, in the young animal and in the older, yellower like old ivory. The buffalo's uterus is whiter, and is stippled with

Table I

*Some comparative measurements of the genitalia of the cow and buffalo**

Part	Cow			Buffalo		
	Means and their standard errors	Standard deviations	Coefficient of variability per cent	Means and their standard errors	Standard deviations	Coefficient of variability per cent
A. Uterus						
Length of horn (greatest curvature)	18·47 ± 1·49 (18)	6·14	33·24	28·48 ± 1·05 (47)	7·23	25·39
Length from internal os to division of horns	10·94 ± 0·50 (25)	2·44	22·30	12·15 ± 0·50 (65)	3·36	27·65
Girth at fundus	7·69 ± 0·46 (8)	1·31	17·04	9·31 ± 0·31 (18)	1·30	13·96
Width at fundus	3·71 ± 0·16 (8)	0·46	12·40	4·50 ± 0·16 (18)	0·69	15·33
B. Fallopian tubes						
Length	16·60 ± 0·73 (16)	2·84	17·11	20·34 ± 0·98 (18)	4·16	20·45
Breadth	0·41 ± 0·04 (16)	0·14	34·87	0·54 ± 0·03 (18)	0·11	20·30
C. Ovary						
Right						
Length	26·37 ± 1·30 (19)	..	..	23·02 ± 0·59 (60)	..	..
Breadth	18·32 ± 1·08 (19)	..	..	17·07 ± 0·56 (60)	..	..
Left						
Length	25·26 ± 1·11 (19)	..	..	22·33 ± 0·45 (59)	..	..
Breadth	16·84 ± 0·86 (19)	..	..	15·84 ± 0·49 (59)	..	..

*A and B were measured in centimeters and C in millimeters. Figures in brackets indicate the number of observations.

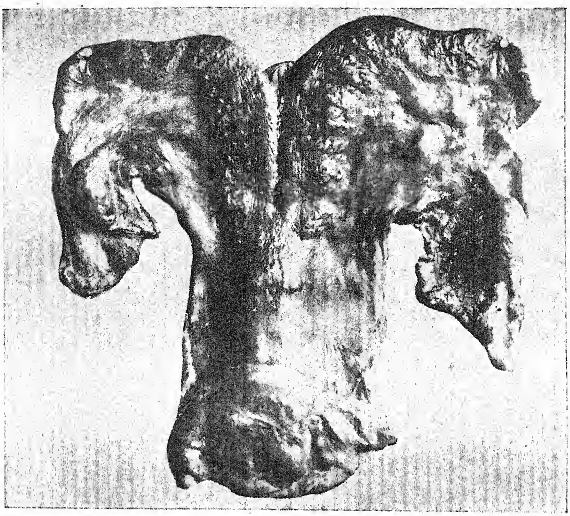


FIG. 1. Uterus of a cow

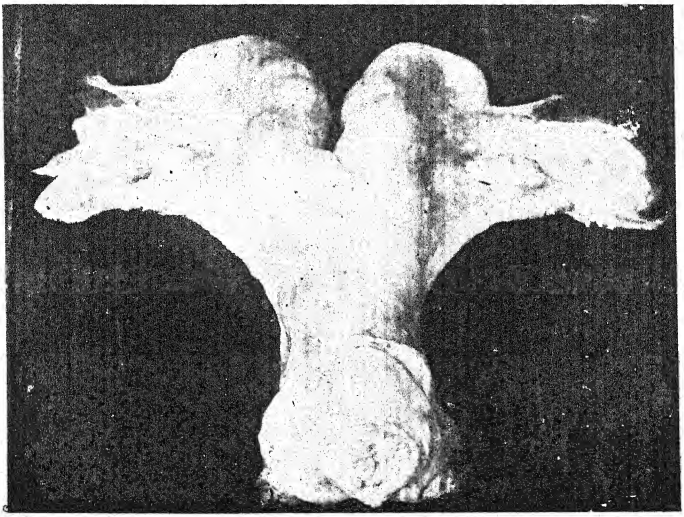


FIG. 2. Uterus of a buffalo

FIG. 1

FIG. 2

FIG. 3

FIG. 4

FIG. 5

FIG. 6

FIG. 7

FIGS. 1-7. Various stages of *corpora lutea* of cow

superficial veins, not obvious in the cow. The surface of the cow's uterus, except near its attachments, is satin-like, but that of the buffalo is faintly knotted both by the above-mentioned veins and by white convoluted striae. Again, a point of importance when making tactile examinations *per rectum*, except in the heifer, the ancestral uterus of the cow is comparatively flaccid and collapsed, but in the buffalo it is turgid and circular in cross-section (Plate VI). However, as the exact age and number of calvings of the animals examined were not known, these are approximate impressions gained from handling numerous organs. A more exact knowledge requires a deeper intimacy with the genital cycle.

Internally, the non-gravid cotyledons do not obviously differ, but it should be mentioned that, in either animal, the appearance of the resting cotyledons is variable. Their apices may be dimpled, flat or rounded. Again, they may be ivory-pink, *cafè-au-lait*, or even dark-brown in colour. In younger animals, they are usually closely spaced, forming deep rugae; in old animals, they may be almost unnoticeable. Williams [1921] and Quinlan [1929] attribute this variation to disease, but we feel that as yet insufficient evidence can be adduced to prove this contention.

Fallopian tubes. The differential measurements of the fallopian tubes are given in Table I. The buffalo's are more deeply involved in the broad ligament than the cow's; they are also coarser in appearance.

Ovaries. It is difficult to compare the sizes of the ovaries for the normal variation within the species is considerable. Some measurements, however, are given in Table I.

Nevertheless, the disposition of the ovaries differs notably, for the cow's lie loosely in their attachments, whilst the buffalo's are more tightly secured. This may be why the buffalo's ovary is more difficult to locate per rectum. The ovaries also differ in appearance; in the cow's the immature follicles are superficial, mottling its surface and suggesting sub-superficial bubbles. The exterior of the buffalo's ovary, however, except where a follicle is maturing, is usually homogeneous.

Ovarian cycle. The changes in the corpus luteum, even of the same species are never alike. Moreover, the colour of the corpus luteum alters when drying. In the colour-plates and description, therefore, an attempt has been made to give an average appearance of the ovarian variation.

In the cow, the about-to-rupture follicle (Plate VII, fig. 1) is a slightly protruding sac, its wall being very thin at its apex. The follicle usually arises on the free curvature but occasionally it lies close to the ovarian attachments (Plate VIII, fig. 5). Wright (unpublished) fixes the mature follicle of the European cow at 14 mm. diameter, while simultaneously various nascent follicles up to 5 mm. diameter appear elsewhere on the surface. After rupture, a blood clot, and then a haemorrhagic corpus luteum, 10-12 mm. diameter occupies the cavity. Natural rupture occurs towards the end of oestrus and the corpus luteum therefore forms during early post-oestrus (two to three days after rupture). Two or three days later the corpus luteum reaches 12 to 15 mm. diameter. Regrettably a specimen of this phase could not be illustrated, but Wright (personal communications) describes the colour as cream, and McNutt [1924] as light brown. At this stage a small portion of luteal substance often protrudes through the aperture of rupture. McNutt [1924] says protrusions are very inconstant; in the present specimens they occurred in 54·5 per cent of 16 mature corpora lutea. Subsequent stages of the luteal cycle are reproduced diagrammatically in Plate VII.

There is some disagreement about the periods of maturation and regression of the corpus luteum, but enlargement is known to last at least 12 and, in the opinion of some, 17 days. However, it seems safe to state that the body and protrusion enlarge rapidly during the first half of the cycle and remain about maximum until the seventeenth day.

In the present work, the average diameter of mature bodies was 20 mm. the protrusion measuring about 5×10 mm. in the early stages and 10×28 mm. at maximum. During maturation, the colour of the corpus luteum changes from stone or buff to bright yellowish orange, but the protrusion often remains haemorrhagic (Plate VII, fig. 2 represents 6 to 8 day body and fig. 3 represents 12 to 17 day body). After conception, the body, apart from losing its haemorrhagic appearance, persists, unchanged both in size and colour, as the corpus luteum of pregnancy. Failing conception, the corpus luteum starts to regress about the seventeenth day or a little earlier. The hæmorrhagic appearance of the protrusion disappears within a week and the entire body becomes yellow (Plate VII, fig. 4). The corpus luteum shrinks and the protrusion becomes overgrown by the adjacent ovarian tissue—a process simulating inward migration (Plate VII, fig. 5). During regression, the colour changes of the corpus luteum are very striking; the yellow first turns to a bright coral or brick-red body about 8 mm. diameter lying wholly within the ovary and later to a small brown scar 3 to 5 mm. diameter (Plate VII, fig. 6). Some ovaries contain numerous coral or brown bodies, but other, quite functional glands, lack them. McNutt [1924] suggests the former are ovaries of aged animals where regression is slow and the latter, the glands of young animals in which regression is always rapid. However, in the present instance it is not safe to dogmatise,

because, failing previous oestra history, the age of a corpus luteum after three weeks can only be guessed by the appearance of further regular ovarian activity; but in historyless slaughterhouse specimens there is nothing to shew whether one or more œstral periods have been missed or, whether an intermediate false heat has occurred.

In the buffalo, the immature follicles are not generally so superficial and the mottling of the surface of the ovary is therefore not often present. The thinning of the apex of the maturing follicle is less noticeable than in the cow. In this work, follicles varied from 5 to 16 mm. diameter, but those easily rupturable ranged from 10 to 16 mm. A maturing, but as yet not superficial follicle on a cut face of the gland, appears at Plate VIII, fig. 1. At Plate VIII, fig. 2, is illustrated a recently and naturally ruptured follicle filled with blood clot and coagulum. Plate VIII, fig. 5 illustrates an unruptured, nearly mature follicle (left extremity of the gland). McNutt [1924] says there is no haemorrhage into the recently ruptured follicle of the cow, but in the buffalo, at least, this appears to occur.

The newly formed corpus luteum is about 13 mm. diameter and is pinkish-grey, veined with red (Plate VIII, fig. 3). This veining, together with a whorling of the luteal substance, gives the cut surface of the corpus luteum a marbled appearance characteristic of the buffalo. At this stage a small haemorrhagic protrusion is usually present. Enlargement up to 20 mm. diameter continues until several days before the next follicle ruptures but, unlike the cow, the greyish-pink veined coloration remains; and it is notable that never during the cycle is the luteal substance yellow. The protrusion is sometimes very large indeed, often larger than the body itself (Plate VIII, fig. 5). In the event of conception, the corpus luteum remains at its maximum (Plate VIII, fig. 9) and the peculiar greyish-green depicted in the plate is not uncommonly encountered, either during pregnancy or when the corpus luteum is mature. As regression occurs, the red-veining of the body and haemorrhage on the protrusion disappears, and the whole turns white (Plate VIII, figs. 6 and 7). Then the protrusion becomes overgrown and the corpus luteum sinks into the stroma. Later the body becomes stone coloured and 5 to 7 mm. diameter (Plate VIII, fig. 8). In the buffalo, luteal protrusions were slightly commoner than in the cow, viz. in 58·8 per cent of 71 observed cases. Their size varied from 3 to 10 mm., the largest being 16 mm. diameter, and their apices were mostly very haemorrhagic. McNutt [1924] mentions central cavities in the corpus luteum of the cow, but such were not observed in those of the Indian zebu. In the buffalo, however, they were relatively common varying from 3 to 5 mm. diameter.

II. Some pathological conditions of the genitalia of Punjab cows and buffaloes

The organs examined in this work were ex-slaughterhouse and presumably a large proportion were animals condemned for sterility. On this account, with the exception of metritis, no figures of lesion incidence have been attempted while inter-species comparison has likewise been avoided.

Metritis. Among the uteri of 45 cows and 185 buffaloes, the mucosae of 15 (33 per cent) and 37 (20 per cent) respectively were congested. Metritis, therefore, is relatively common in the Punjab cattle.

Two common types of metritis were distinguished —a chronic and an acute. In the chronic type, the lining of the whole organ was furred and greyish-cream in colour. The uterine wall was flaccid and without tone, often with a milky exudate on the mucosa. Occasionally patches of congestion occurred, especially in the tips of the horns; but, while uncommon here, they were even rarer in the uterine body. The cotyledons, however, were uniformly suppressed. In almost all cases, the ovaries were a functional, and the stroma of the gland was homogeneous and fibrous. This condition would produce irrevocable sterility and was more frequent in the cow.

In acute metritis, the uterine wall was more or less tonic, and the furring of the mucosa was lacking. Congestion, however, was much more prevalent, ranging from small patches in the tips of one or both horns to widespread inflammation. The amount of exudate varied with the degree of congestion: it was cream to coffee coloured and sometimes very sticky. The apices of the cotyledons were generally more congested than other parts and were often slightly necrotic. The ovaries were usually functional and normal, but in a few one or other had become fibrous and afunctional, resembling those of the 'chronic' uterus.

Richter [1926] says, 53 per cent of sterilities in Europe, are due to metritis; Webster [1932] in New Zealand gives 5·4 per cent. In the present work the ratio of metritis to total disease was 33 per cent in cows and 22 per cent in buffaloes. Whether ovarian dysfunction precedes metritis, producing an easily invaded uterus, or whether bacterial invasion is primary and ovarian dysfunction secondary, is debatable. As far as acute metritis is concerned, the writers prefer the latter view, for, although, cervicitis is perhaps uncommon in India, in many of the metritis cases examined the ovaries were apparently normal. Failing exudate and congestion in a 'chronic' uterus, the ovaries being functionless, it is difficult to decide whether the lesion is primary, or the result of improper uterine stimulation. It is peculiar that congestion is most common in the uterine horns, farthest from external contamination, but perhaps a more stagnant drain-

FIG. 1

FIG. 2

FIG. 3

FIG. 4

FIG. 5

FIG. 6

FIG. 7

FIG. 8

FIG. 9

FIGS. 1-9. Various stages of *corpora lutea* of a buffalo

[*Indian J. vet. Sci.*, Vol. 15, Part III

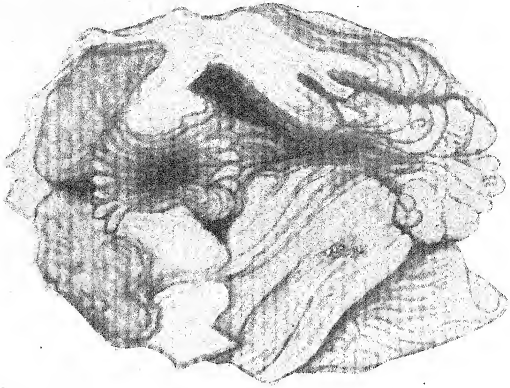


FIG. 1. Cervicitis (acute)

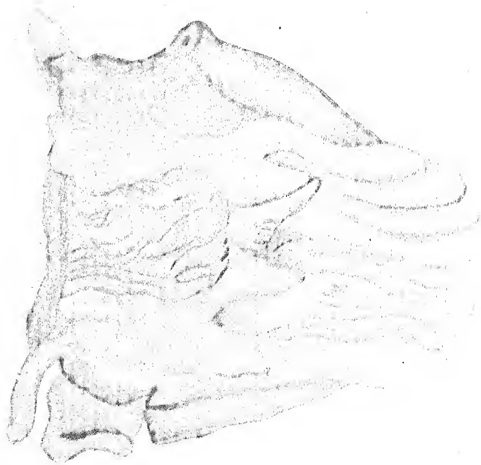


FIG. 2. Cervicitis (sub-acute)

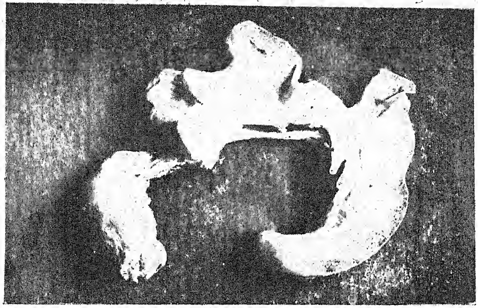


FIG. 1.

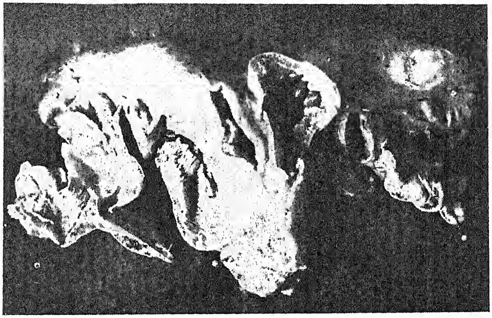


FIG. 2.

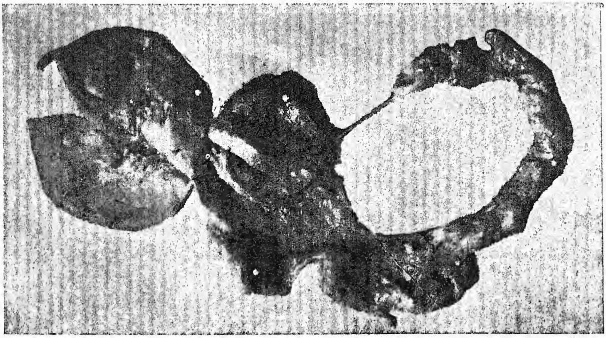


FIG. 3.
FIGS. 1—3. Pneumosalpinx

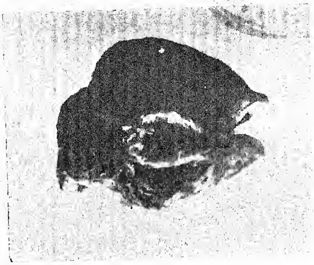


FIG. 1. Thin-walled luteal body

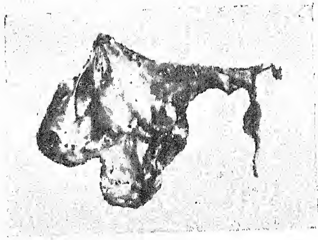
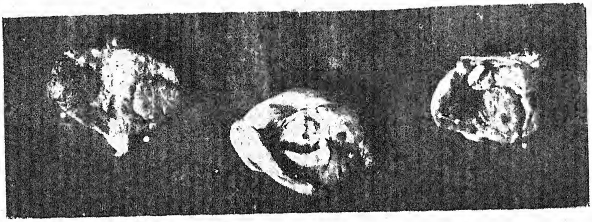
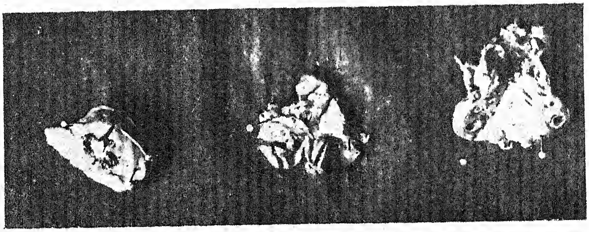


FIG. 2. Cystic degeneration of ovary



A B C

FIG. 3. A, B and C. Dermoids



A B C

FIG. 4. A, B and C. Cystic *corpora lutea*

age causes persistent infection hereabouts and not elsewhere.

Two cases of pyometra have been noted in the zebu; the uteri contained three or four pints of milky fluid; the uterine walls were distended, thin and atonic, whilst the mucosa, especially of the horns, was deeply congested and in places necrotic. In one the cervix also was severely inflamed (Plate IX, fig. 2). The ovaries of both contained central cavities probably cysts. Nielson [1926] contends that retained corpora lutea occur only secondarily, resulting from chronic, pyometric endometritis. In these specimens corpora lutea were not retained.

A single case of tubercular metritis was found in a buffalo. The uterine wall contained hard, caseous, pea-sized nodules, packed with acid-fast baccilli. The nodules might have been palpable per rectum.

Cervicitis. Few cases of cervicitis were seen. The only outstanding example is illustrated at Plate IX. More frequently a few streaks and patches of slight congestion were observed but, because the butchers severed the organ near the cervix, these may have been artifacts.

Abnormalities of the fallopian tubes. Simple salpingitis was not seen in the cow, but was twice found in the buffalo. The tubes were hardened and thickened suggesting chronicity. In one the attendant ovary was embedded in the broad ligament and, together with the opposite ovary, was afunctional. Five other fallopian lesions were found in the buffalo; in all, one or both tubes were blind at the ovarian end. In four the uteri were congested, their horn tips being indurated and cartilaginous with the lumen constricted (2-3 mm. diameter) and tortuous. The induration formed a separable core in the horn tip. Ovarian abnormalities occurred in four of these specimens. Williams [1921] describing salpingitis says that as the disease progresses, the pavilion is always involved and may be closed, either by adhesions within the funnel or with the ovary; the ovary may then become encapsulated in the ovarian pocket, and the tube distended with fluid. Williams denotes the condition as 'hydrosalpinx'. He believes the lesion results from metritis and associates it with cystic corpora lutea.

The authors' specimens agree with Williams' description as to the occurrence of metritis in four, luteal cysts in one and encapsulation of the ovary in two cases. But, in all five, no obvious inflammation, either past or present could be detected in the tubes; the blind ends were usually attached to the ovary normally whilst, on resection, they were lined by apparently normal fimbriæ. This is unlike Quinlan's [1929] description who writes of 'elongated mucous folds occluding the tube and forming septa'. Further, whilst in hydrosalpinx the tubes contain fluid, in four of the present specimens they held gas, which could not be evacuated into the uterus by pressure. The inflated portion resembled, in miniature, a child's toy sausage balloon, and pneumosalpinx seems a more descriptive name.

Three specimens of this lesion were preserved and are illustrated in Plate X. In one the distension (bottom right, Plate X, fig. 1) was $34 \times 1{\cdot}5$ cm., both ovaries were normal and functional, but the last quarter of the uterine horn on the affected side was indurated (bottom left, Plate X, fig. 1). In the second specimen both tubes were affected, the left terminating in a gas-filled sac like a small walnut (bottom left, Plate X, fig. 2). The encapsulated left ovary contained immature follicles. The right uninflated tube was also blind, its attendant ovary being functional but encapsulated (the resected ovaries can be distinguished in Plate X, fig. 2, inside the curvature of the horns). Metritis was also present. The blind end of the left tube of the third specimen was attached to the ovary by a fibrous cord (right centre, Plate X, fig. 3). The tip of the horn was indurated and contained a core (extreme left, Plate X, fig. 3). This tube contained fluid not gas. The attendant encapsulated ovary was fibrous and afunctional and contained a cavity about 7 mm. diameter. (Plate X, fig. 3, centre). The right ovary was functional. Of the two unillustrated specimens, the left tubes only were affected; in one the left ovary was afunctional, the right continued a cystic corpus luteum; in the other the left ovary was afunctional, but the right was normal.

Ovarian abnormalities

(a) *Abnormal disposition of the ovary.* In the buffalo but not in the cow, the encapsulation of the ovary varying from a partial enclosure, where ova might still be discharged, to a total encapsulation was common. In some ovaries, exposed parts were crossed and recrossed with fibrous cords. In all but one of these ovaries, corpora lutea indicated function, but in one (Plate XI, fig. 1) a luteal body consisted of a thin-walled cavity. Whether (this ovarian encapsulation resulted from infection of the fallopian tubes is problematical, because the tubes of three of the cases were macroscopically unchanged.

(b) *Dermoid tumours.* Teratomata were recorded in four buffalo ovaries. Three were small pea-like bodies with a hard shell enclosing a dense coagulum, sometimes comingled with hairs (Plate XI, figs. 3, A and C). The fourth was like a small walnut. In three cases the ovaries were functional; in a fourth (unillustrated) the ovarian tissue was almost replaced by a cyst. Nevertheless, the little remaining tissue had functioned recently

(c) *Cystic degeneration of the ovary.* Three ovarian cystic degenerations were observed: all in the buffalo. In specimen 1 (Plate XI, fig. 2) multiple cysts entirely replaced the left ovary. The right ovary was normal and functional. In specimen 2 (Plate XI, fig. 3 B, right hand extremity) the anterior part of the ovary consisted of a single sac (25 mm. diameter) containing coagulum. The sheath of this cyst was continuous with the ovarian covering; the ovary was nearly intact, but its stroma contained a central cavity 4 mm. in diameter. The third case simulated the second.

(d) *Cysts of the corpus luteum.* Luteal cysts were noted in the cow and in the buffalo. Many corpora lutea contain small central cavities (about 3 mm. diameter) appearing as a parting between the whorls of substance. It is difficult therefore to define normality. In some of these cases, however, the luteal body was thinly walled cavity frequently containing a whitish coagulum. Table II gives some comparative sizes of corpora lutea and their cavities. Luteal cysts are to be seen in Plate XI, Figs 4, A and C. In Plate XI, fig. 4 B, a white central coagulum can be observed.

Table II

Comparative sizes of corpus lutea and their cavities

Size of corpus luteum	Size of cyst
12 mm.	6 mm.
6 ,,	4 ,,
15 ,,	13 ,,
21 ,,	15 ,,
12 ,,	9 ,,
10 ,,	5 ,,

This condition, it appears, is often associated with other ovarian or tubular disease, e.g. embedded and cystic ovaries.

Attempts were made to express cystic and normal corpora lutea of buffaloes, but, except where the natural protrusion was large, expression during life without injury to the ovary did not seem feasible.

Cysts on other parts of the genitalia. Cysts on buffalo's genitalia are very common on the uterine wall, on the broad ligament, on the tubes or near the ovaries. They vary from the size of a pea to a Brazil-nut, but do not appear to be of great importance and their aetiology is unknown.

Summary of part I

The macroscopic appearance of the genitalia of the cow and buffalo differs considerably. The uterus of the buffalo is larger and more turgid than the cow's. It also has a peculiar blue and white surface stippling. The fallopian tubes of this animal are coarser and more embedded in the broad ligaments. The cow's ovaries are mottled and freely mobile in a loose attachment, but the buffalo's have a plain white surface and are more tightly secured. The luteal cycle in the cow is notable for its bright colour changes, whereas in the buffalo, apart from hæmorrhage on the protrusion and a remarkable red-veining, colour changes are almost absent, and the background is always a subdued greyish stone-white.

Summary of part II

Two common types of metritis have been described, chronic and acute. The former was more prevalent in the cow. Two cases of pyometra in the cow and a case of tubercular metritis in the buffalo were noted.

Cervicitis was rarely encountered in the buffalo or cow but one case has been described and illustrated.

Abnormalities of the fallopian tubes were not seen in the cow. But several were described in the buffalo, five being unusual.

Ovarian abnormalities were rare in the cow, but were relatively common in the buffalo. Especially noteworthy were ovarian encapsulation, teratomata, and cysts of the reproductive organs.

Acknowledgements

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BLOOD CHANGES IN CATTLE FED ON A PROTEIN-DEFICIENT DIET

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Deficiency in the supply of protein is one of the chief limiting factors in formulating balanced rations. According to Wright's [1937] estimate, only 0·079 lb. of digestible crude protein is available per day for an animal weighing 500 lb. as compared with the normal requirements of 0·3 lb. This deficiency is aggravated in times of famine and war. During the first great war, oedema, which has been ascribed by Knack and Neumann [1917], Kohman [1920] and Maver [1920] to lack of protein in the diet, occurred among the famine-stricken population of Central Europe [Peters and Van Slyke, 1932]. Krishnan [1944] also observed it during the 1943 Bengal famine. In our experimental animals, oedema of the feet and eyelids was observed when they had been kept on a protein-deficient ration for about three months.

In view of the common prevalence of protein deficiency in livestock in India and the lack of information on blood changes which could be used for the diagnosis of this condition, a systematic study was made of the various blood components of cattle maintained on a low-nitrogen ration for a considerable period as compared with those of cattle on a normal ration.

Details of the low-nitrogen and the nitrogen-free rations used are given in a previous article [Kehar, Mukherjee and Sen, 1943]. Nine Hariana bullocks were maintained on a low-nitrogen ration for 29 days and subsequently on a nitrogen-free ration for 24 days. Prior to this, the animals had been kept on a sub-maintenance level of protein allowance for about two months. The control animals were fed on a balanced ration. Animals of both groups were adults and apparently in good health. Blood was drawn by jugular puncture after 12 hours' fasting. The following morphological and chemical constituents were studied: Total red cells, total and differential white cell counts, cell volume, hæmoglobin, iron, sugar, cholesterol, inorganic phosphorus, calcium, magnesium, total nitrogen, non-protein nitrogen and the protein fractions. All the morphological constituents were studied by Napier and Das Gupta's [1941] methods. Observations were made in duplicate and the average values are given in Tables I to III.

Table I

Blood constituents in normal and protein-deficient bullocks

	Normal animals							Protein-deficient animals									
	1	2	3	4	5	6	Average	1	2	3	4	5	6	7	8	9	Average
Red cells (millions per c. mm.)	7·5	5·8	9·8	9·1	7·7	6·0	7·7	8·4	8·0	5·9	6·2	6·7	6·6	5·8	6·6	6·2	6·18
White cells (thousand per c. mm.)	5·7	6·8	9·2	9·7	7·7	7·5	7·7	7·7	8·0	7·2	6·8	6·8	7·2	6·1	7·3	6·9	7·15
White cells per cent distribution—																	
Lymphocytes	66	65	64	69	51	64	63·1	63	62	63	58	60	..	..	..	..	61·2
Monocytes	9·5	11	2	3	2	10	6·2	9	10	4	3	7	..	..	..	..	6·6
Neutrophiles	20·5	20	32	27	44	21	27·4	20	20·5	26	28	25	..	..	..	..	23·9
Esinophiles	3·5	4	2	1	3	4·5	3·0	8	7·5	7	10	8	..	..	..	..	8·1
Basophiles	0·5	0	0	0	0	0·5	0·16	0	0	0	1	0	..	..	..	..	0·2
Cell volume per cent	54	50	43	50	57	50	50·6	58	44	44	49	40	37	47	47	43	45·4
Mean corpuscular volume (μ μ)	72·0	86·2	43·8	53·2	74·0	83·3	68·7	69·0	55·0	74·6	79·0	59·7	56·1	81·0	71·2	69·3	66·1
Mean corpuscular hæmoglobin γγ	14·5	17·4	10·2	12·4	15·4	19·2	14·9	11·78	10·37	16·7	15·0	12·2	12·27	14·65	13·63	13·87	13·39
Mean corpuscular hæmoglobin concentration.	20·2	20·2	23·2	23·4	20·9	23·0	21·8	17·06	18·86	22·5	18·97	20·5	21·89	18·08	19·14	20·0	19·66

Red cells. The average red cell count in the control group (Table I) was 7·7 millions (minimum 5·8; maximum 9·8), while that in the protein-deficient group was 6·48 millions (minimum 5·8; maximum 8·4) per c.mm. These observations are in conformity with those of Weech *et al* [1937] on dogs and of Kyer and Bethal [1938] on rats, though Orten and Orten [1940] found the erythrocyte count to be nearly normal in rats fed on a low nitrogen diet from the time of weaning up to 100 days of age.

White cells. The average white cell count was 7·7 thousands per c. mm. (minimum 5·7; maximum 9·7) in normal animals as compared with 7·14 thousands (minimum 6·4; maximum 8·0) in the low-protein group (Table I).

White cell per cent distribution. From a perusal of the differential leucocytic counts in Table I, it appears that there is no significant difference between the normal and the protein-deficient group except in eosinophiles. Ordinarily, it might indicate helminthiasis, but repeated examinations of the faeces of the animals revealed no helminth ova.

Cell volume. The average cell volume per cent (Table I) was 50·6 (minimum 43; maximum 57) in the normal animals and 45·4 (minimum 37; maximum 58) in the protein-deficient animals. The results obtained by Shelburne [1934] and Weech *et al* [1937] in dogs, Kyer and Bethall [1938] in rats are in agreement with our findings on bullocks.

Corpuscular values. The average values for mean corpuscular volume, mean corpuscular hæmoglobin, and mean corpuscular hæmoglobin concentration (Table I) are 68·7 Cu µ (minimum 43·8; maximum 86·2), 14·9 γγ (minimum 10·2; maximum 19·2) and 21·8 per cent. (minimum 20·2; maximum 23·4) respectively in the normal animals and 66·1 Cu µ (minimum 55; maximum 81·0); 13·39 γγ (minimum 10·37; maximum 16·7) and 19·66 per cent (minimum 17·06; maximum 22·5) in the protein-deficient animals.

Sugar. Blood sugar was determined by Host and Hatlehost's [1920] method. Average values for the control group, as shown in Table II, are 75·6 mg. per cent (minimum 68·8; maximum 81·1) as compared with 70·0 mg. (minimum 67·1; maximum 72·4) in the protein-deficient group. Russell and Weber [1934] found no significant difference in the sugar contents of the blood of two groups of hens receiving diets containing 12·7 and 19·1 per cent of protein respectively. Wang *et al.* [1930-31], however, found a rise from 82·5 to 86·9 mg. per cent when normal young women were kept on a low-nitrogen diet.

Haemoglobin. For the estimation of hæmoglobin Newcomer's [1919, 1923] technique was followed. The average haemoglobin content (Table II) was lower in the protein-deficient group. These findings are in conformity with those of Weech *et al.* [1937] on dogs, Kyer and Bethal [1938] on pregnant rats and Orten and Orten [1940] on growing rats, McCance *et al.* [1938] on women, and Mackay *et al.* [1942] on men, women and children.

Iron. The iron content of the blood, as estimated by the method of Wong [1928], was 34·2 (minimum 30·2; maximum 39·2) and 40·2 (minimum 33·2; maximum 45·2) mg. per cent in the portein-deficient and control groups respectively (Table II).

TABLE II

Constituents of blood and serum in normal and protein-deficient bullocks

—	Normal animals							Protein deficient animals									
	1	2	3	4	5	6	Average	1	2	3	4	5	6	7	8	9	Average
Whole blood (mg. per 100c.c.)																	
Sugar	75·9	79·4	81·1	77·6	68·8	70·6	75·6	72·4	70·6	67·1	68·8	68·8	68·8	68·8	72·4	72·4	70·0
Haemoglobin	10·9	10·1	10·0	11·7	11·9	11·5	11·0	9·9	8·3	9·9	9·3	8·2	8·4	8·5	9·0	8·6	8·9
Iron	45·2	35·2	33·2	42·6	43·0	42·2	40·2	32·5	30·2	31·2	33·4	39·2	38·6	..	..	..	34·2
Total cholesterol	..	..	190	182	195	177	186	121	114	109	110	125	111	..	..	..	115
Serum (mg. per 100 c.c.)																	
Calcium	11·2	11·0	10·0	11·0	9·6	9·4	10·4	8·6	9·9	8·2	10·2	9·9	10·3	10·4	10·3	10·0	9·7
Magnesium	2·86	2·63	2·73	3·06	2·78	2·61	2·78	..	3·15	1·76	2·91	2·46	2·70	2·75	2·04	2·52	2·51
Inorganic phosphorus	3·96	3·92	6·20	4·36	3·39	3·70	4·26	4·79	5·67	4·15	3·85	3·91	4·05	3·32	3·56	3·75	4·11

Total cholesterol. For cholesterol estimation the technique described by Banerji [1933] was followed. The average values for cholesterol (Table II) fell from 186 mg. per cent in the control group to 115 mg. in the low protein group. Shelburne [1934] and Page *et al.* [1937], however, found that the change from normal to low protein diet had no consistent effect on the cholesterol content of the blood.

Calcium. Serum calcium was determined by Clark and Collip's [1925] modification of Kramer and Tisdall's [1921] method. The average values for serum calcium as shown in Table II are 10·4 (minimum 9·4 ; maximum 11·2) mg. per cent in the control group and 9·7 (minimum 8·2 ; maximum 10·3) mg. per cent in the protein-deficient group. Similar results were obtained by Wang *et al.* [1930-31] with young women on low-nitrogen diet.

Magnesium. Serum magnesium was estimated by the method of Fiske and Subbarow [1925] as modified by Holzapfel [1934]. Average values for the control group were 2·78 (minimum 2·61 ; maximum 3·06) and 2·54 (minimum 1·76 ; maximum 3·15) mg. per cent for the protein-deficient group.

Inorganic phosphorus. Serum inorganic phosphorus was estimated by the method of Fiske and Subbarow [1925]. Average values for the control and protein-deficient groups are 4·26 mg. per cent (minimum 3·70 ; maximum 6·20) and 4·11 (minimum 3·32 ; maximum 5·67) mg. per cent respectively.

Protein fractions. Serum protein fractions were estimated by the method devised by Campbell and Hanna [1937] with slight modifications adopted by Kehar *et al.* [1940].

TABLE III

Serum-protein fractions in normal and protein-deficient bullocks (gm. per cent)

—	Normal animals					Protein-deficient animals									
	1	2	3	4	Average	1	2	3	4	5	6	7	8	9	Average
Total protein	8·25	9·19	8·56	8·44	8·61	6·30	7·50	6·30	7·96	7·56	7·38	7·56	7·50	7·06	7·23
Non-protein nitrogen (mg. per cent)	35·5	36·5	32·9	36·4	35·3	..	27·2	20·8	25·7	25·1	29·5	30·2	..	..	26·4
Englobulin	0·90	1·00	1·00	1·21	1·03	..	0·50	0·80	0·80	0·81	0·88	0·75	0·89	0·93	0·80
Pseudoglobulin I	2·80	3·06	2·84	2·69	2·85	..	2·50	1·80	2·94	2·59	2·59	2·83	2·50	2·43	2·53
Pseudoglobulin II	0·19	0·32	0·16	0·20	0·22	..	0·50	0·40	0·26	0·16	0·16	0·24	0·11	0·19	0·25
Total globulin	3·89	4·38	4·00	4·10	4·09	..	3·50	3·00	4·00	3·56	3·63	3·82	3·50	3·55	3·57
Albumin	4·36	4·81	4·56	4·34	4·52	..	4·00	3·30	3·96	4·00	3·75	3·74	4·00	4·51	3·91
Albumin / Globulin	1·12	1·10	1·14	1·06	1·11	..	1·14	1·1	0·99	1·12	1·03	0·98	1·14	0·99	1·06

It will be observed from Table III that there is a general drop in the average values of total protein and all the protein fractions except pseudoglobulin II in the protein-deficient group, as compared with the control group. These observations regarding the decrease in values of total protein and albumin are in conformity with those of Frisch *et al.* [1929], Weech *et al.* [1933], Shelburne [1934], Bloomfield [1935] and Page *et al.* [1937]. Bloomfield [1933, 1935] maintained that in rats on a diet containing 10 per cent protein an initial fall of about 10 per cent occurs in the total serum protein, but that value remains almost steady even though the protein fraction of the diet is further reduced. Frisch *et al.* [1929], however, showed that serum protein values of rats dropped as low as 3·6 per cent on a low-protein diet and rose to 6·9 when casein was added to the diet. Weech *et al.* [1933] and Shelburne [1934] pointed out that increase or decrease in serum globulin was not consistent in dogs fed on a low protein diet.

The non-protein nitrogen value in the control group was 35·3 (minimum 32·9 ; maximum 36·5) and 26·4 (minimum 20·8 ; maximum 30·2) in the protein-deficient group. Russell and Weber [1934] and Sas [1935] showed that in hens and dogs the non-protein nitrogen remained unchanged when the subjects were on a protein-deficient ration. But Wang *et al.* [1930-31] found that the non-protein nitrogen of the blood of young women decreased when they were transferred to a low protein diet. Knack and Neuman [1917] pointed out that the non-protein nitrogen fell as the rate of nitrogen catabolism diminished.

It will be observed that the average values for total globulin was 4·09 (minimum 3·89 ; maximum 4·38) in the normal animals and 3·57 (minimum 3·00 ; maximum 4·00) mg. per cent. in the protein-deficient animals. A careful perusal of the available literature revealed no previous record of the variations in globulin fractions as a result of a protein-deficient diet. It will be seen from Table III that

both the euglobulin and pseudoglobulin I fractions are less in the protein-deficient than in the control group. Since globulins are associated with the maintenance of body integrity, it may be that the decreased amounts of these immunologically active fractions in protein deficiency render the animals more susceptible to infection.

SUMMARY

Observations on the morphological and chemical constituents of the blood of normal cattle and of those fed on a protein-deficient diet are presented. It has been found that the red cells, white cells, cell volume, sugar, haemoglobin, iron, total cholesterol, calcium, inorganic phosphorus, magnesium, non-protein nitrogen and the protein fractions are decreased in protein-deficient animals. As the globulins are especially associated with the integrity of the body cells, it may be that the decreased amounts of these immunologically active fractions in protein-deficient animals render them more susceptible to infection.

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REPORT ON A DETAILED STUDY OF METHODS OF YOKING BULLOCKS FOR AGRICULTURAL WORK PRESENTED TO THE IMPERIAL COUNCIL OF AGRICULTURAL RESEARCH

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(With two text-figures).

THIS is a report of a study which is part of a larger programme of research being carried out at the Allahabad Agricultural Institute on the methods of yoking bullocks for agricultural work and on the effect of body conformation on draft ability of work animals. This report will deal with the study of yoking methods, yokes and harness.

The main object of the whole scheme was to secure information on the effect body conformation has on the draft ability of work cattle. This information, especially if a positive correlation were found, should provide a basis for selecting breeding animals. If readily measured or recognised body measurements can be determined which are consistently associated with high draft ability, they should be a valuable guide to the animal breeder in selecting breeding stock. We wished to determine how far any such measurements associated with draft ability coincided with body measurements or proportions known to be consistent with milk yielding ability. We particularly

cularly wished to determine how far simple measurements, which could be made by ordinary breeders without elaborate apparatus or much knowledge of genetics, could serve as a guide to the selection of breeding stock. We believed that the results of such a study, even if negative, would be of value. It would show that some basis of selection other than such measurements is necessary. Information as to the extent to which a certain body conformation is desirable or necessary for maximum draft ability would help to settle the controversy over whether it is possible to have a dual purpose breed or not.

If we were to test the draft ability of the animal, it seemed necessary that the animal be tested with a yoke or harness which enabled it to exert its maximum strength. There are widely differing types of yokes in use in India which appear to differ in the extent to which they allow the animal to use his strength. Aside from its bearing on this scheme, information about the effect of the yoke on the ability of a pair of animals to pull a load would be useful in practice. If yokes really differ as much as they appear to, there should be marked advantage in selecting the best one for general use. The adoption of a yoke which would increase the power delivered 10 per cent would be equivalent to adding the power of about 5,500,000 work animals to the power available to the Indian farmer. (There are 55,794,864 bovine work animals in India—British India and the Indian States taken together,—according to the 1940 census of live stock). It seems desirable therefore to carry out tests to determine the best type of yoke or harness before trying to test animals. It is with this preliminary work on methods of yoking that this report is concerned.

Some further statement of the importance of the problem studied may be of value. The Indian farmer is practically entirely dependent on the draft bullock or buffalo for power used in farm operations. The power developed by a single pair of bullocks is small but the aggregate of all power used on the farms of India is very large. If the average power developed per animal is as little as one-fourth horse power (H.P.), the aggregate is nearly 14,000,000 H.P.

It seems unlikely that animal power will be displaced in the near future in India. The small size of individual holdings, the poverty of the individual farmer, the dense population, the lack of fuel oil and other factors are all against the extensive introduction of mechanical power for the work now done by bovine animal power. Even if mechanical power is to replace animal power, the period intervening before this is likely to take place on any large scale seems likely to be long enough to justify study of the best method of application of animal power.

It is well recognised that the Indian farmer is hampered by lack of power. One of the most common excuses for not accepting better implements, particularly ploughs, is the supposed inability of the bullocks to deliver the required power. The only effective way to increase the productive power of the farmer to any great extent is by enabling him to do more work, or better work, in a given time. One of the most effective ways to do this is to put into his control more power per worker. Without any essential change in the present system of cultivation, the provision of more power will improve the position of the farmer by enabling him to do better work, to do more work, and so to increase his earnings. This improvement will be in addition to anything which may be accomplished by better seeds and better farm practices. In fact most better farm practices ultimately depend on better implements and more power to work them. At present, Indian work cattle seem to develop less power than should be expected of them. Aside from any question of improving the bullock, any improvement in the yoke used which will enable the bullock to better apply his strength will be a gain.

REVIEW OF THE LITERATURE

Very little testing of draft animals has been done anywhere in the world, so the literature on the subject is scanty. Rule of thumb methods have developed various types of harness and of yokes in different parts of the world and even in different parts of a country, as in India. Very few of these seem to have been subjected to scientific study and none seem to have been developed as the result of study and systematic tests of different types.

It is known that draft ability in both horses and bovine animals is roughly proportional to body weight, that the big animal in general can pull more than the small. A good deal is known by farmers in western countries about the fitting of collars to horses, for instance, and there are references in the literature to the sore shoulders resulting from poorly fitted collars or other causes. Occasional references can be found to the advantages of a 'well-fitted yoke'. Our study has not uncovered a single comparative study of the fitting or design of either yokes or harness. No article has come to our attention discussing the subject as the main item of the article.

It is not difficult to understand reasons for this situation. The art of using animals for draft work is very old and the main types of harness and of yokes were fixed long before the age of scientific enquiry. When men first began in modern times to question all sorts of practices and ideas, perhaps the harness or the yokes in use functioned relatively better than other things. Attention therefore tended to be focussed where most difficulty was felt, on the implements used with the animals. Except for the appearance of galls and sores there was no very sensitive method of judging the fatigue of the

animal. By the time that the spirit of enquiry had spread sufficiently and the numbers of research workers had increased to the point where such problems might have had more serious attention, the development of the internal combustion engine, applied particularly to the tractor, had progressed to the stage where it seemed likely to displace animal power in agriculture. If animal power was to be displaced, there seemed little value in studying it or in trying to improve it or its application. This probably largely accounts for the scarcity of literature on the subject.

A few efforts have been traced to improve yokes in India. Krishnamurty of the Madras Veterinary College designed an improved yoke but it was not included in the types of yokes reported in common use in Madras by the Director of Agriculture. Similarly, Mushtaq Ahmad of the Punjab Veterinary College designed an improved yoke which was not included in the types reported by the Director of Agriculture, Punjab, in response to our enquiries for designs to be tested. A 'yoke' or wooden collar was designed for a single bullock at Poona Agricultural College and was included in the types submitted by the Director of Agriculture, Bombay, and a similar yoke with no reference to origin was included in those reported used in Gwalior. Charley at Coimbatore designed a leather strap harness for a single bullock, described in leaflet No. 85 of the Department of Agriculture, Madras. No publication which has come to attention gives any information on comparative usefulness of any of these, the published material being confined to descriptions and to the uses to which they may be put. In some cases, authors make claims of superiority but do not cite tests to prove the claims. No foreign publication has come to our attention from anywhere in the world dealing with tests of harness or yokes. The field therefore seems to be a completely virgin one, unexplored and apparently practically undiscovered.

The literature on the testing of animals is scant but not quite non-existent. Bulletin 240, October 1926, of the Agricultural Experiment Station, Iowa State College of Agriculture and Mechanic Arts, by Collins and Caine, devotes 28 pages to 'Testing Draft Horses'. This is the only publication in English devoted primarily to discussing tests of draft horses which has come to our attention.

'Investigation of the Working Performance of Swiss Cattle' by Hans Wenger, Bern, 1939, is the only study of the draft ability of bovine animals which has been available during this study. It is in German and no English translation has been found. Wenger lists a bibliography of 58 items, only three being in English, one a work on statistical methods, on the bulletin by Collins and Caine referred to above and the third a study by Brody of Missouri University. From a translation of the index and summary, it does not appear to contain anything on the question of harness or yoking nor does the bibliography refer to any publication on the subject.

With such a scarcity of work done on draft power, it is not surprising that there is little material available on methods and equipment for testing draft power. Wenger gives a detailed description of the loading car which he used, which is of the general type described by several other workers, particularly Collins and Caine.

Collins and Caine in Iowa State Bulletin 240 describe three variations of a traction dynamometer car for testing animals as follows :

'In the first dynamometer, built for this work, provision was made for hitching each of two horses to weights in a manner similar to that shown in fig. 2. In this case the tractive pull resulting from lifting the weights is more than enough to propel the dynamometer and it is necessary to apply some resistance to hold the machine back. This was done by gearing the rear wheels to a rotary pump and then regulating the discharge from the pump to produce the desired resistance. This regulation was made automatic by connecting the valve so that it is operated by a change in the height of the weights relative to the frame.'

'When the weights are at the bottom the valve is closed and the rear wheels are locked. As the weights are lifted the valve gradually opens until at the top it is wide open and the pump furnishes a minimum of resistance. In action this automatic control allows the dynamometer to move just fast enough to keep the weights suspended whenever the team is moving. The condition of the road surface or the grade does not affect the amount of work done by the team but merely requires that more or less resistance must be furnished by the pump. The tractive pull exerted by the team is constant and equal to the sum of the weights which are held suspended.'

'In action, then, the tractive force required to move the dynamometer (variable) plus the resistance furnished by the pump (governed by position of weights) is equal to the tractive pull exerted by the team (constant). The dynamometer, of course, has practical limits. On slippery roads it is difficult to get enough traction for the wheels, in which case the weights would not be lifted and the team would be exerting a tractive force something less than the weights. On the other hand where the roads are extremely soft or a very steep hill is encountered, it is quite possible that the tractive pull required to move the machine itself will be greater than the amount of the weights. This may readily occur if only a light load were applied. In such a case the weights would strike the top of their guides and the team would be exerting a greater tractive pull than the amount of the weights.'

'The dynamometer as built was equipped to furnish a tractive resistance for each horse which could be varied from 60 to 350 pounds.'

'In order to test horses for their maximum effort for short periods, a larger machine was built. This dynamometer is shown in figs. 5 and 6. This operates in essentially the same way as the smaller machine just described but differs in constructional details and in capacity. In the construction of the machine, the chassis of a Nash quad four-wheel drive truck was used. The engine of the truck was replaced with a rotary pump so that the same train of gearing could be used to drive the pump. The team in this case is hitched to a double tree and only one set of weights is used. The weights are made of reinforced concrete and normally rest upon the frame as shown. Each of the weights may be attached to the weight beam by turning a lock on the beam. The valve controlling the discharge from the pump is so connected to the weight beam that the valve is closed when the beam is at the top. By this means the forward movement of the truck is regulated automatically so that the weights will always be lifted while the dynamometer is in motion. This machine has a maximum capacity of 4,100 pounds tractive pull and a total weight of 10,000 pounds.'

'The machine shown in figs. 7 and 8 was also built for testing horses for their maximum effort. It is mounted on the rear of a Ford truck chassis and as the engine was not removed, the truck can be readily transported with its own power. The large weights used are cast iron disks weighing 250 pounds each. As many of these as desired may be attached to the lifting cable. Small increments of weight may be added by placing them in the space provided between the two top weights. As shown in the diagram (fig. 7) a pulley is so connected to the cables that 500 pounds tractive effort is required on the hitch to support each 250 pounds weight. This was necessary in order to keep the total weight of the machine as low as possible. A special sprocket attaches to the rear of the worm shaft and drives the pump by means of a roller chain. The lever operating the pump discharge-valve is connected to the weights, so that the valve is closed when the weights are resting on the frame and wide open when the weights are at the top of their guides. By this means, just enough resistance is automatically applied at all times to the forward movement of the dynamometer to keep the weights suspended. The height at which the weights are carried depends upon the speed of the team, the amount of weights attached and the road surface, but is of no importance as long as the weights are not touching either the top or bottom of the guides.'

'This machine has a maximum capacity of 3,200 pounds tractive pull and a total weight of 5,230 pounds. In order to get enough traction for average road conditions, it is necessary to use skid chains on the rear wheels and to put a skid under the front wheels as shown in fig. 8. For loads very near the maximum, additional weight is applied to the truck body. This is usually accomplished by getting men to stand on the platform.'

'For transportation it is only necessary to disconnect the tongue and skids, load them on the platform and take off the roller chain which drives the pump.'

'This type of machine has been further improved by the addition of a three-speed auxiliary transmission with power take off attachment. This provides six forward speeds for transporting the machine, and the power take off provides a more suitable method of driving the pump; otherwise no important changes have been made. The machine is illustrated in fig. 9.'

It will be noted that the first dynamometer car described by Collins and Caine and those described by European workers are designed to give working loads. The two latter described by Collins and Caine are designed primarily to test maximum draft ability, though by suitable adjustment it is possible to use them for smaller loads also.

Broady and fellow workers used a tread mill apparatus instead of a loading car, which they have described in Missouri Bulletins 209 and 383. The principle of the tread mill is that the backward push of the animal's feet tends to move the travelling belt on which the animal stands. This allows the animal to stand in one place, which may be under a shelter. In this way it is possible to have a uniform unvarying footing on which the animal works and which is independent of weather conditions. They first experimented with loading the animal by using the tread mill to drive an oil pump in the same way as was used on the Iowa loading car by Collins and Caine. This proved unsatisfactory, possibly due to the tread mill being in bad mechanical repair. They later loaded the animal by making it lift weights.

M. A. Sharp described a simple traction dynamometer for maximum draft tests in Vol. 22, No. 1, dated January 1941, of the Agricultural Engineering Journal. This consists essentially of a two-wheeled cart made from the back axle of a lorry or car with a platform and draft rig added to it. It also carries a roller, on which a rope can be wound, fitted with brake drums and brakes, which, when tightened, resist the rotation of the roller. The roller is also geared to a device, such as a car jack, which can be used to progressively set the brakes as the roller revolves. Rotation of the roller is secured by unwinding the rope, the other end of which is fastened to a tree or stake. The apparatus is prepared for a test by winding the rope on the roller with the brakes slack and the team or single animal hitched to the

car. As the animal moves forward, the rope is pulled off the roller, setting the brakes and increasing the resistance to rotation of the roller. As the car can only move forward as the rope is allowed to unwind from the roller, increasing the resistance to rotation of the roller also increases the resistance to forward movement of the car. The animal or animals continue to go forward till the resistance of the car equals the effort they can or will exert under the degree of urging applied. In this case the pull rises continuously to a maximum and must be measured by some indicating or recording device interposed in the hitch from the animals to the car.

There is somewhat more literature on recording apparatus for measuring draft. Most commercial apparatus available is very expensive and not available in India and difficult to import in wartime. Even if available, none of it seemed entirely satisfactory in one or another respect. The greatest drawbacks, however, were non-availability and cost. Collins describes the Iowa traction integrating dynamometer. This instrument is fairly suitable for use with some types of apparatus but did not seem suitable for our purpose, in addition to being unavailable in a reasonable time. The bibliography at the end of this report gives references to various articles on recording apparatus.

Experimental procedure

The decision as to which type of apparatus to use was difficult. It was finally decided to use two types of apparatus for the different parts of the experiment: Sharp's simple dynamometer car for the testing of yokes and a tread mill for the testing of animals. This choice was partly dictated by the fact that the dynamometer car could be cheaply and quickly made available while the tread mill had to be constructed and would take time. The tread mill type of apparatus was chosen for the latter study of effect of body conformation on draft ability because it facilitated the use of certain auxiliary apparatus which will be discussed in the report on that part of the study and because it made standardization of conditions easy.

The lack of any satisfactory recording type of apparatus made it necessary to choose an indicating method of measuring the draft. Not even an indicating dynamometer scale was available, so a large Salter's spring balance was adapted to the purpose by adding a second pointer which would record the maximum pull exerted in any one test. While this has worked reasonably well, it is hoped that a recording unit will be available in the second part of the study.

The choice of just what to measure was even more difficult than how to measure it. It was recognised that animals do not, when doing ordinary work, continuously exert the maximum power of which they are capable. A commonly accepted standard for loading horses is that the ordinary draft should not exceed one-tenth of the live weight. The Physiological Chemist in the Scientific Reports of the Imperial Institute of Agricultural Research, Pusa, 1933-34, in reporting some tests carried out at Pusa, says, 'In these tests the draft has been one-fifth to one-sixth (of the body weight) and yet the animals did not show any suffering.'

What we wanted to know was the maximum load which a given animal could pull in ordinary kinds of work for ordinary work periods, day after day. Theoretically, this meant a load which would result in a state of fatigue at the end of the work period, beyond which it would be undesirable or dangerous to go. We found no method of testing the state of fatigue and no standard maximum state of fatigue which an animal could bear. It is known of course that if an animal or a man works beyond his normal capacity, he loses condition, becomes leaner and eventually may develop other symptoms, or even collapse entirely, if the overloading is carried to great extremes. Such effects ordinarily only show up after long periods, too long at least for us to use them for purposes of this research. Various methods, such as blood analyses, were suggested as 'possibly' offering a means of determining fatigue. These did not seem suitable because there was no standardized procedure or any correlation known between the results of the analysis and the degree of fatigue. To have tried to work out such standards would have taken more time than was available. Collins and Caine say 'It is practically impossible at present to determine the state of fatigue of an animal. Experienced horsemen may observe the general condition of an individual but they cannot determine the actual physical condition and know exactly if the horse is approaching a state of fatigue. In two years of experimental work where horses were pulling loads equivalent to a horse power or more for eight hours per day, day after day, they showed no outward signs of a fatigued condition;....'. Since we found no end point which could be definitely determined, in tests using loads comparable to ordinary working loads, it was necessary to choose something for our tests which could be definitely determined. We realized that we do not yet know exactly the relation between the maximum load an animal or pair of animals can pull and the load they should be expected to pull day after day. Horse pulling contests and other testing in America has shown however that, in general, pulling contests are generally won—that is, maximum pulls are made—by animals which are known to be good workers. We therefore feel justified in assuming that in bullocks also, there is a relation between maximum load pulled and draft ability as generally applied under ordinary working conditions.

The applicability of the maximum pull to testing of yokes seems even more definite. Presumably, ability to pull a given load will be limited by one of two things, the strength of the muscles or the unit pressure under the yoke on the neck. The unit pressure under the yoke as the result of a given draft load will depend on the shape of the yoke and of the animal's neck—on the fit of the yoke to the neck. Tests of yoke to determine whether a difference in the area of contact enables a bullock to pull more or not seems to be one valid test of yokes. If it can be determined that changes in the yoke do not result in increased load pulled, it seems valid to assume that the load pulled is limited by the muscular power which can be applied by the animal. Since we were not able to find any alternative test which seemed to offer any advantage and since the results to be secured by tests of maximum pulling ability seemed theoretically valid, we feel justified in using tests of maximum load pulled in both parts of the scheme.

It is desirable, however, that tests be carried out to determine the fraction of the maximum pull which can be consistently pulled by working animals. While as stated* above, one tenth of the live weight is generally accepted as a suitable standard for horses, there is little or no experimental evidence to support it, and the information we have seems to throw doubt on it as a standard for bullocks. Desirable as it may be to have the information, it is outside the scope of this research problem.

As a preliminary step in the procedure, requests were sent to the Director of Agriculture of each of the provinces and of the larger Indian States known to have organized Departments of Agriculture, asking for dimensioned sketches of the typical yokes in use in their respective areas. These requests brought replies with drawings. These drawings were studied to determine what variation there was among them, and 15 types were selected as representing all the differences likely to in any way affect the usefulness of the yoke. Others appeared to be different only in ornamentation or other similar unimportant details. To these we added the 'Nagpuri' yoke in use at the Institute and one made to dimensions from the Almora hill district, making a total of 15 yokes selected for later testing. Two other yokes were made and tested to check certain points, making a total of 17 yokes actually tested. A yoke was made to the design of Krishnamurty of Madras Veterinary College. This is primarily a cart yoke and it was found impracticable to test it with the apparatus we were using. As it did not seem to have any advantage likely to justify further attempts to test it, it was excluded from the tests. Sketches of these yokes are shown in Fig. 1 and Fig. 2.

* Bulletin No. 240, Iowa State College of Agriculture and Mechanical Arts, p. 219.

EXPERIMENTAL DATA AND RESULTS

For the testing of the yokes, five pairs of bullocks were selected from the work animals regularly in use on the Institute Farm. A ploughman accustomed to handling the bullocks was also selected from the farm workmen and all tests were driven by the one man. Whenever he was off duty for any reason, testing was suspended and the staff was occupied in other duties connected with the research. All tests reported were done with the original five pairs of bullocks. On the few occasions when any one animal was indisposed for any reason, it was always possible to carry on with another pair. No major illness or injury to any of the test bullocks occurred during the period of the tests.

The tests were carried out by Mr P. K. Bhargava and Mr B. K. Mukerjee. Generally both were on duty when tests were under way, and readings were confirmed by both. On a few occasions, when it was necessary for one of them to be absent, the other carried on the testing alone. These occasions were few.

Testing was started with yokes Nos. 1, 2, 3 and 4, which were first ready. The procedure was to first accustom the animals to the apparatus and to the new yoke when a new yoke was taken in hand. Then 25 pulls with each pair on a yoke was made before the next was taken in hand. When the full number of tests on one group of yokes was completed with one pair of bullocks, the other pairs were used in turn till the whole group of yokes had been tested by all five pairs. In the meantime, another group of yokes, Nos. 5, 6, 7 and 8 were made ready and tests carried out. In this way the first 15 yokes were tested a total of 1,875 observations.

During these tests, certain difficulties were met. The bullocks were totally unaccustomed to the particular type of work and to the slight noise the machine made. They were only accustomed to the ordinary field work of the farm and to the ordinary methods of driving, and it was found difficult to control them in any uniform manner. Sometimes they tended to move quite fast and at other times they required considerable urging. It was difficult to apply a uniform degree of urging so that they put out the same degree of effort every time. They tended to learn that they could only go so far before the machine stopped them and to be quite willing to stop when they had reached what they estimated to be the right place. This was partly overcome by driving them in different directions and by the changing of the testing from place to place. Some interruption was caused by rain on certain days and it was necessary to suspend testing for some days towards the end of the rains because the footing was unfavourable when the ground became very wet and covered with slime.

As we neared the end of the first series of tests, an attempt was made to analyse the results. The results were averaged, plotted in various ways and studied statistically. There appeared to be quite significant differences between the pairs of bullocks, but the differences between the yokes were not statistically significant. Study of the data seemed to indicate that it did not prove the lack of difference between yokes, but rather that our testing had not been under sufficiently controlled conditions, particularly with regard to the handling of the bullocks. The data showed too great difference between the high and low readings, indicating that in some cases the bullocks had exerted abnormal effort and that in others they had shirked. Results of every test had been recorded in an attempt to avoid anything which might resemble 'doctoring' the datta. The effect was somewhat that of including in a series of analyses for nitrogen, samples into which had flies in the course of the digestion.

Whether to include any given test or not presented a real difficulty. Obviously, when the bullocks ran and came up at the end of the test with a jerk, the maximum pull would be abnormal and it was easy to discard such test. Tests when the bullocks shirked were more difficult. Determining the standard of urging to be used was not easy. It might vary all the way from whispered commands to severe beating. This seemed to be the biggest variable and the most difficult to control. It seemed definite that we should not determine which data to record by the magnitude of the reading. It was decided therefore, after consulting with Dr Sukhatme, Statistician of the Imperial Council of Agricultural Research, to again run through the tests, using a fewer number of trials of each yoke and attempting to control the conditions more carefully. We decided to try to determine which trials to discard before reading the dynamometer scale by carefully watching the animals during the test.

We therefore randomized the order in which the yokes were to be tested and ran the whole 15 again, making only five tests on each yoke and using one pair of bullocks. When this series of tests was completed and the results were studied, it appeared that they were more consistent than the first series. To make sure, we again ran a similar series of tests, with a new randomization order, making five tests each. Three such replications were made to see if we could duplicate the results. The results of the replications were consistent, showing only the variation which would be reasonable when working with animals. The results of the three replications of five tests with each pair of bullocks were combined,

Table I

Average pull of bullock with double yokes

Bullocks	Pull in pounds for each yoke (Average of 15 observations)																		
Team No.	Weight in lb.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	Average
34 & 35 . .	1,515	812	847	885	815	622	841	775	775	810	808	804	799	788	739	791	823	745	793
15 & 24 . .	1,795	919	899	890	945	688	909	892	911	884	931	931	882	879	898	913	856	814	885
32 & 36 . .	1,455	872	902	908	920	737	926	857	924	882	870	898	889	877	893	901	847	805	877
3 & 4	1,625	923	953	919	885	824	800	836	894	875	857	873	837	880	910	881	859	809	866
13 & 14 . .	1,425	833	752	791	775	735	797	768	767	760	716	845	769	771	806	773	779	737	775
Average pull per yoke	..	872	861	879	868	721	854	826	853	842	836	870	835	839	849	852	852	782	..

and an average taken for each pair with each yoke was calculated. Table I gives these averages of 15 tests with each yoke and each pair.

While the yokes are classifiable into definitely different groups, the difference as shown by these tests is not large. The second group gave pulls averaging about 98 per cent as great as the average pull of the first group or 97 per cent of the pull given

Statistical analysis of the above results

Table II

Analysis of variance for the double yokes

Due to	D.f.	S.S.	M.S.	F by calculation	F from table		Level of significance
					5%	1%	
Bullocks	4	2,674,114·2	668,528·5	41·31	2·52	3·65	1%
Yokes	16	1,669,996·2	106,249·8	6·59	1·81	2·32	1%
Interaction	64	1,030,531·2	16,102·1	12·31	1·26	1·38	1%
Residue	1190	1,546,247·2	1,299·4				
Totals .	1274	6,950,888·8					

Grouping of yokes

I III V VII VIII

3, 1, 11, 4, 6, 8, 15, 2, 14, 9, 13, 10, 12, 16, 7, 17, 5

II IV VI

by the best yoke, No. 3. The other groups gave pulls 96 per cent, 95 per cent, 89 per cent and 82·5 per cent as large as the average of the best group. These relations should be understood to hold only for the conditions under which the tests were made. It is probable that some yokes, particularly No. 16, would show up worse under actual working conditions of long continued work than it did under the short time tests actually made.

Table III gives comparative measurements of the different yokes, and Figures 1 and 2 show the difference in designs of the yokes. Table III also indicates the area from which each yoke design came. No. 1 is the ordinary 'Nagpur' yoke in use at the Institute for several years and originally modified from a yoke brought to the Institute from Nagpur, C. P. Nos. 13 and 14 are modifications of the same yoke in an attempt to get smaller and larger areas of contact between neck and yoke. No. 5 was made to the design of Mushtaq Ahmed as an 'improved' yoke. No. 17 was modification of the 'improved' yoke to Madras design.

These measurements do not give any very clear clue to why one yoke is better or worse than another. The two worst yokes according to these tests showed the largest and the smallest areas in contact with the neck. The measurement of the area in contact with the neck was perhaps the least satisfactory measurement made. Various methods were tried and none was found very satisfactory. The method finally adopted was to smear the yoke with lamp black mixed with oil; then the yoke was carefully placed on the neck and pressed firmly down and back to get an impression. The yoke was then removed and placed on the ground, and a soft copper wire was bent to fit the shape of the impression. The wire shape was then carefully transferred to a sheet of graph paper and the area carefully determined by counting the squares. The areas so determined are only approximate. There is some reason to believe that the area recorded for No. 5 is high, but this did not invalidate the results as will be shown later. The weight of the yoke also does not appear to be a factor, at least within the limits of the weights of the yokes

tested. The best group included the heaviest and some of the lighter yokes while the lightest yoke is near the bottom of the list. If anything, the evidence would be in favour of a heavy yoke. No other factor stands out as definitely explaining the superiority of one yoke over another.

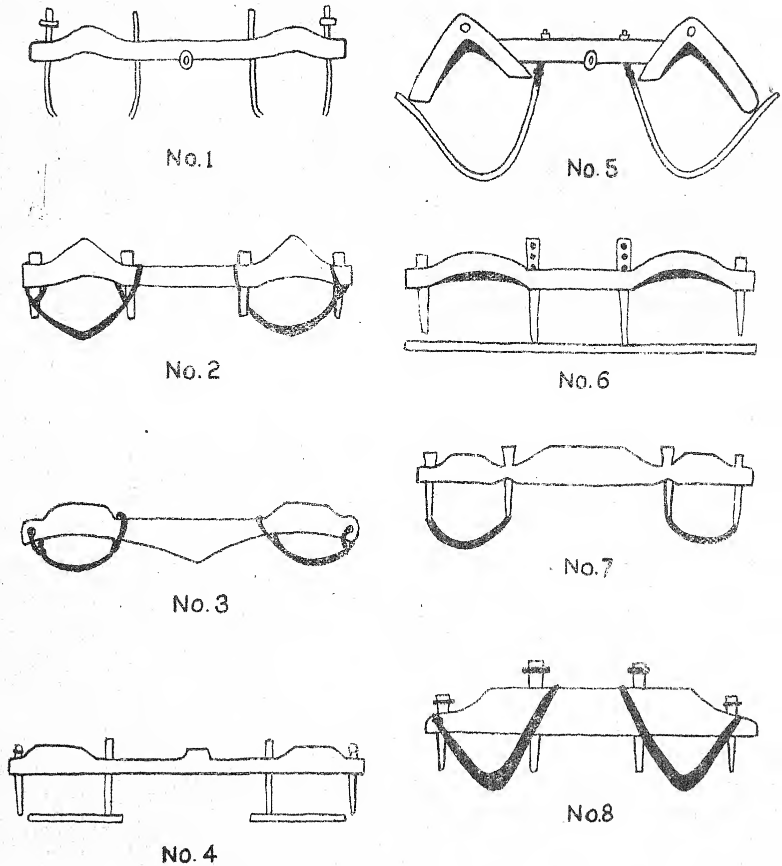

Fig. 1. Sketches of yokes

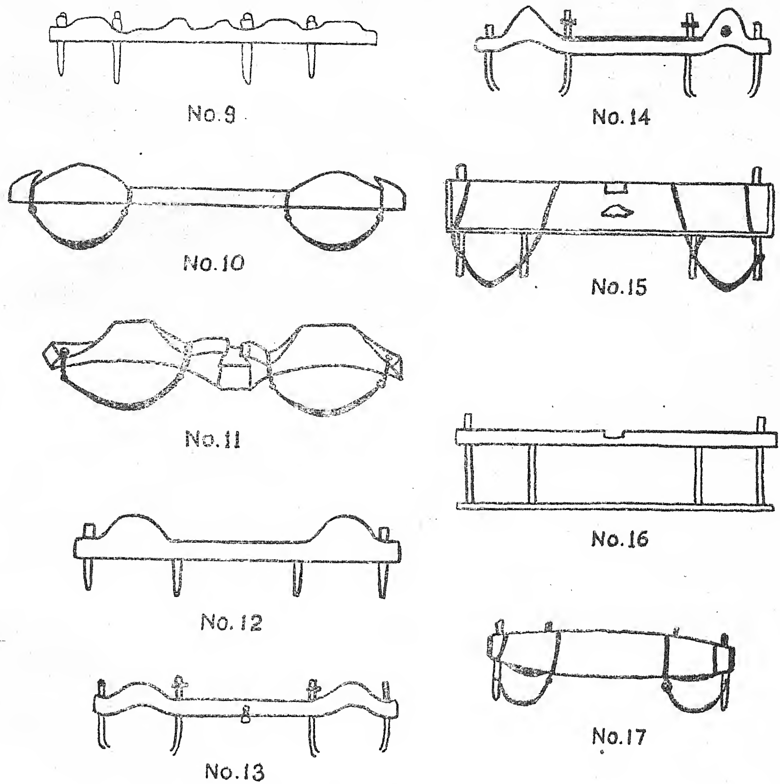

FIG. 2. Sketches of yokes

TESTS OF SINGLE BULLOCK HITCHES

After completing the tests of yokes for pairs, it seemed desirable to investigate the usefulness of single animal hitches. In the replies from the Directors of Agriculture, Gwalior and Bombay had described essentially the same single bullock yoke of wood. Charley's leather harness had come to attention otherwise. In anticipation of these tests, an American horse collar had been imported and a similar collar had been made locally. A hitch known as a 'birh' is in common use in the area of Allahabad for hitching a single bullock in front of a cart when three animals are required, or for drawing a hand cart when only one animal is used. The imported collar was found to be too big for use on the animals available for tests and so was discarded. In studying the results of the tests for yokes for pairs, it appeared that probably the poor results from the tests of yoke No. 5 was due to the fact that it fixed the distance between the bullocks quite rigidly, allowing them very little freedom of individual movement and that

TABLE III

Details of yokes construction

Yoke No.	Province where yoke is used	Weight of yoke in lb.	Average pull in lb.	Wood used in making yoke	Length of yoke in ft. and in.	Width of yoke at neck position—in.	Length of yoke at neck position—in.	Thickness of yoke at neck position—in.	Depth of curve of yoke at neck position—in.	Average area of contact between yoke and neck—sq. in. (10 bullocks).
No. 1	U. P.	26 with pipe	872	Babul	4-5	7	16	$1\frac{1}{2}$	H=$2\frac{3}{4}$" V=*nil*	52·138
No. 2	Assam	27	851	Sakhu	4-$6\frac{1}{2}$	7	12	$3\frac{1}{2}$	H=*nil* V=$\frac{1}{4}$"	42·965
No. 3	C. P.	24	880	Sakhu	4-6	$4\frac{1}{4}$	11	3	H=$2\frac{1}{4}$" V=2"	42·58
No. 4	Punjab	30	869	Sakhu	5-0	5	11	3	H=*nil* V=*nil*	40·455
No. 5	Punjab	30	721	Babul	4-8	5	9	3	H=*nil* V=$8\frac{3}{4}$"	60·112
No. 6	Punjab	26	862	Babul	4-7	$4\frac{1}{2}$	13	5	H=*nil* V=$1\frac{3}{4}$"	41·166
No. 7	Kashmere	16	826	Sakhu	4-6	$4\frac{1}{2}$	12	$2\frac{1}{2}$	H=*nil* V=*nil*	37·554
No. 8	C. P. East Circle	25	853	Babul	4-$6\frac{1}{2}$	4	13	13	H=*nil* V=*nil*	38·59
No. 9	Punjab	22	842	Sal	4-9	6	$9\frac{1}{2}$	$2\frac{1}{2}$	H=*nil* V=*nil*	39·215
No. 10	Bihar	28	836	Sal	6-3	$6\frac{3}{4}$	17	3	H=*nil* V=*nil*	46·885
No. 11	C. P.	35	870	Sal	4-4	$7\frac{1}{2}$	$14\frac{1}{2}$	3	H=1·1/8" V=1·5/8"	56·625
No. 12	Punjab Country type	20	834	Sal	4-$9\frac{1}{2}$	$6\frac{1}{2}$	9	3	H=*nil* V=*nil*	40·261
No. 13	U. P.	18 with pipe	839	Babul	4-3	$4\frac{1}{2}$	12	$2\frac{1}{2}$	H=$1\frac{3}{4}$" V=$1\frac{1}{2}$"	40·01

TABLE III—*contd.*

Details of yokes construction—contd.

Yoke No.	Province where yoke is used	Weight of yoke in lb.	Average pull in lb.	Wood used in making yoke	Length of yoke in ft. and in.	Width of yoke at neck position—in.	Length of yoke at neck position—in.	Thickness of yoke at neck position—in.	Depth of curve of yoke at neck position—in.	Average area of contact between yoke and neck—sq. in. (10 bullocks).
No. 14	U. P. .	32 with pipe	849	Sakhu .	4-5	9	12	3	H=1″ V=2½″	59·86
No. 15	U. P. Hills	20	852	Sakhu .	5-0	6	11	2	H=*nil* V=*nil*	45·87
No. 16	Local .	20	832	Sakhu .	4-9	3	10	Round	*nil*	35·36
No. 17	Madras Improved Cart yoke	35	782	Sakhu .	3-10½	4	7	Round	*nil*	28·246

the design of the part in contact with the neck was not in itself bad. In order to investigate this and to give an additional single animal hitch, one end of the No. 5 yoke was disconnected and modified slightly to enable it to be used as a single hitch. This gave a total of 5 hitches, suitable for single bullocks, to be tested.

Essentially the same procedure was used with the single hitches as with the pair yokes. The same cart was modified by fitting shafts instead of a single beam. Five single bullocks were chosen, from among the 5 pairs used for the previous tests, and trained to work single. Replicated series of 5 tests were made with each bullock of the five on each of the hitches. Table IV gives the results of the tests of the single hitches and the analysis of the data.

The above data is interesting not only because it gives an idea of the relative values of the different hitches but because of the indication it gives of the relative efficiencies of working of single bullocks and of the same animals hitched in pairs by the use of a common yoke.

The study of the pull possible with single hitches has two possible utilities. The possible desirability of using single animals instead of pairs in some circumstances and the question of whether some form of harness hitch with an equalizing bar behind—a so-called double tree—might give more effective pulling power for pairs. The results with Mushtaq Ahmad's improved yoke indicates the undesirability of too rigidly restraining the bullocks against sidewise movement. While the ordinary yoke gives more freedom than does the Mushtaq Ahmad yoke, it gives less than would be possible with a harness and double tree hitch. This needs further investigation. If 1/6th of the body weight or 1/4th of the maximum pull, two figures which seem to be roughly equal in the data from these tests, should prove to be reasonable loading, the draft ability of the bullocks tested would come approximately within the range of loads commonly required or imposed by ordinary small implements, 100 to 120 lb. pull. The practicability and desirability of using single animals of a slightly larger size instead of a pair of very small animals on the smaller farms needs to be investigated further.

The reason, or at least one reason, why the Poona-Gwalior yoke performed poorly on single animals seemed to be at least partly because of the poor fit on the neck of the bullocks used and because it bore on the point of the shoulder joints. It appears to have been designed for animals having a much thicker neck than the test animals had. Bullocks differ from horses in having a very thin covering of muscle and flesh over the shoulder joint and pressure on the joint seems to be uncomfortable to the bullock, while it is precisely here that the horse does its pulling. Part of the superiority of the half of the Punjab yoke

seemed to be that it did not come down to the shoulder and was narrow at the top.

CONCLUSIONS

1. Yokes of the general type represented by types 1, 3, 4, 11 are definitely better than some others, especially types 12, 16, 7, 17, 5.

2. Yokes which definitely restrict side movement of the bullocks are undesirable.

3. Except for the restraint of side movement, no very clearly defined reason for the superiority of one yoke over another has appeared from these studies.

4. The training and control of the bullocks very greatly affect the results.

TABLE IV

Data of tests of single bullocks hitches

Bullocks	Pull in pounds with each yoke (Average of 30 observations)						Average for bullocks
Team No.	Weight in pounds	Birh	American modified	Punjab	Madras harness	Gwalior yoke	
34	760	611	589	579	485	450	544
35	820	464	438	467	418	445	446
15	910	651	631	609	510	523	585
24	826	617	570	570	519	491	553
14	780	618	591	618	574	529	586
Average for yokes		592	564	569	501	489	

PRELIMINARY STUDIES ON CALCIUM AND PHOSPHORUS IN MILK AND FEEDS OF SAHIWAL COWS

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(With 12 text-figures)

HART *et al.* [1908] observed that when calcium and phosphorus were deficient in quantity in food, the skeletal tissues appeared to be the ready source of supply for milk secreted and there was considerable output of these elements from the gut. If conditions of negative balances continued for a long time a slow shrinkage in milk flow results, but there was no physiological disturbance. In their later observations [1920], they reported a serious effect on reproduction. Even though Forbes *et al.* [1916-18] in their experiments provided for an ample intake of calcium and phosphorus in rich diet containing

alfalfa hay and salts, larger amounts were eliminated than were ingested. Meigs, Blatherwick, and Cary [1919] found that dry pregnant cows were not assimilating calcium from calcium-rich rations, but were actually transferring calcium salts from their skeleton for building the foetal skeleton. Hart, Steenbock and Hoppert [1921] observed that in dry and milking goats calcium assimilation was greater when green instead of dry oats were fed. Cod liver oil (5-10 c.c. per day) consistently changed a negative to a positive balance. Hart *et al.* [1922] found that high-yielding cows were maintained in positive calcium and phosphorus balance on dry alfalfa hay, corn silage and grain mixture. Their subsequent findings [1922], in which timothy hay was used, are not in harmony with the observations quoted above. Bone-meal supplement did not bring about positive balance. Forbes *et al.* [1922] found that the calcium of the bones was more readily available for purposes of milk elaboration than the calcium of the rations and the mineral supplement. They came to the conclusion that calcium metabolism was characterized by its rapid loss from the body during early period of lactation and its retention during the dry period with most rapid storage at the end of the period of gestation. At the time of high milk production, if the food was sufficient to maintain the life of the cow, she would produce milk, even though this involved an extensive drain upon the tissues of her body. Hart *et al.* [1922-23] took up the work again and found phosphorus equilibrium was maintained without, and calcium equilibrium with, bone-meal and green grass supplement.

The cows of the Sahiwal herd at our dairy farm were reported to be licking earth while grazing. As the cows were fed with protein-rich concentrates, green fodder and mineral mixture as 'Churn Flour' this habit of theirs could not be explained. The earth was analysed, but nothing particular was discovered, which could account for this peculiar habit and it was suspected that probably more calcium and phosphorus were excreted by our cows than they were ingesting from the feeds. The present investigation was undertaken to ascertain if there was variation in the percentage ratio and total output of calcium and phosphorus in relation to the yield of milk, age and stage of lactation in the case of the Sahiwal breed of cows and if so to what extent. The above factors in relation to dry and green fodder were also examined.

EXPERIMENTAL

Preliminary experiments were carried out to study the suitability of methods of analysis and to ascertain (i) whether there was any variation in the percentage of calcium and phosphorus in the different milkings of the day and whether these differed from that found in a composite sample of the day's milk, (ii) whether the total amount of calcium and phosphorus in all four milkings differed from that calculated from the composite sample, (iii) whether the ratio $CaO : P_2O_5$ was constant, and (iv) whether there was any change in the percentage of calcium and phosphorus in the day-to-day milk.

For this purpose, three high and three low milking cows, first calver, second calver and adult, all in the same stages of lactation (8th week), were selected. Samples of milk were taken from all the four milkings and composite samples of the milk was prepared by taking 5 c.c. per lb.

The milk was ashed by Neumann's method [1902-03] which was found quite satisfactory. Ten c.c. milk was taken out with a pipette in a 100 c.c. Kjeldahl flask. A separate aliquot of 10 c.c. was weighed in a tared weighing bottle. For calculation, the average weight of 10 c.c. of milk was used, as the difference in day-to-day weight was not very much. But before calving 10 c.c. of milk was weighed separately each day. The milk was ashed with 5 c.c. concentrated sulphuric acid and nitric acid and the solution made up to 100 c.c.

Twenty-five c.c. aliquot was taken and calcium estimated by Kraut's method [1856]. Ten c.c. aliquot was taken and phosphorus estimated by Pemberton's method [1882].

These preliminary experiments revealed that (1) the percentage of these elements in samples of the different milkings of the day did not vary much among themselves or from the composite sample of the day, (2) the percentage of these elements in day-to-day samples of the milk did not differ much and (3) the ratio of $CaO : P_2O_5$ also did not show much variation.

For comprehensive studies, 12 high-milking and 12 low-milking cows of different ages and in the various stages of lactation were selected. The lactation of some of these cows commenced before they calved. Samples of milk were taken once a day at 9 a.m. milking and analysed. The concentrates as well as green and fresh fodders were also analysed for calcium and phosphorus, the latter at an interval of ten days.

One pound of mustard cake per cow was also given from 1 to 10 August and half a pound of boiled linseed per cow was given to those which were in the first 100 days of lactation.

In addition to these concentrates, the cows were taking 20 lb. of green fodder (cowpeas and *jowar* mixture), three pounds of berseem hay and three pounds of *bhusa*. While grazing, they could take about 20 pounds of grass.

The grains, oil cakes, berseem hay and *bhusa* were analysed for calcium and phosphorus. The results are given in Table IV.

The calcium and phosphorus contents of the cowpeas and *jowar* mixed fodder and grass determined at different periods were found to be as shown in Table V.

TABLE I

Names and particulars of cows under experiment

1st Calver	2nd Calver	Adult	Period of lactation
High milkers			
Felbir	Narajkhati	....	From precalving onwards
Nashurki	Enarji	Narbila	First 100 days
	Sakila	Raj Khati	
		Lakholmi	
Tashkam	Lanchasari	....	Second 100 days
	Lakhroochi	Rajsam	....
Low milkers			
Taroochi	Sarajan	....	Second 100 days
Folgai	Lakhrajan	....	
	Rajudhina	....	
Tambutia	....	Rajrama	Third 100 days
Lereng	....	Choreng	
Lakhrami	....	Raj Lari	
		Raj Suri	

TABLE II

Amount of concentrates given to cows in lb. per head per day

Names of cows	26-31 July	1-10 August	11-20 August	21-22 August	Names of cows	26-31 July	1-10 August	11-20 August	21-22 August
High milkers					*Low milkers*—contd.				
Felbir	7	9	11	12	Sarajan	10	10	9	8
Narajkhati	5	7	13	14	Lanchasari	14	14	14	14
Nashurki	15	15	15	15	Lakhroochi	15	14	13	13
Tashkam	16	16	15	15	Narbilla	17	17	16	16
Enarji	17	17	17	16	Rajkhati	17	16	16	16
Sakila	15	15	15	15	Lakholmi	16	14	15	15
					Rajsam	15	15	15	15
Low milkers					Lakhrajan	11	11	9	9
Rajsuri	6	6	6	5	Tambutia	6	6	5	5
Rajrama	9	8	7	6	Lereng	5	5	5	5
Choreng	7	7	6	6	Lakrami	7	7	6	5
Rajudhina	3	2	2	2	Taroochi	12	11	11	11
Rajlari	3	3	3	2	Folgai	12	11	11	10

The composition of the concentrate rations was as given in Table III.

TABLE III

Composition of the concentrate rations

26 July to 4 August		5 to 20 August		21 to 22 August	
Oats	35 per cent	Oats	45 per cent	Oats	35 per cent
Chuni (mixture of pulses)	30 „ „	*Chuni* (mixture of pulses)	35 „ „	*Chuni* (mixture of pulses)	25 „ „
Wheat bran	15 „ „	Wheat bran	15 „ „	Wheat bran	15 „ „
Cotton seed meal	20 „ „	Linseed cake	5 „ „	Groundnut cake	15 „ „
				Linseed cake	5 „ „
				Cotton seed meal	5 „ „
	100		100		100

TABLE IV

Calcium and phosphorus content of the grains, oil cakes, berseem hay, and bhusa

—	CaO per cent	P_2O_5 per cent	—	CaO per cent	P_2O_5 per cent
Oats	0·106	0·284	Wheat bran	0·213	2·112
Gram	0·278	0·642	Linseed cake	0·600	0·744
Cowpeas	0·112	0·754	Mustard cake	1·137	1·154
Arhar	0·134	0·381	Groundnut cake	0·134	2·440
Guar	0·286	0·920	Linseed	0·327	0·795
Cotton seed meal	0·325	1·136	Berseem hay	3·112	0·183
			Bhusa	0·685	0·094

TABLE V

Calcium and phosphorus contents of cowpeas and jowar *mixed fodder and grass*

Period	Cowpeas and *jowar* mixed		Grass	
	CaO per cent	P_2O_5 per cent	CaO per cent	P_2O_5 per cent
26 July to 4 August	0·264	0·109	0·193	0·183
4 to 14 August	0·271	0·116	0·200	0·202
15 to 22 August	0·268	0·112	0·196	0·191

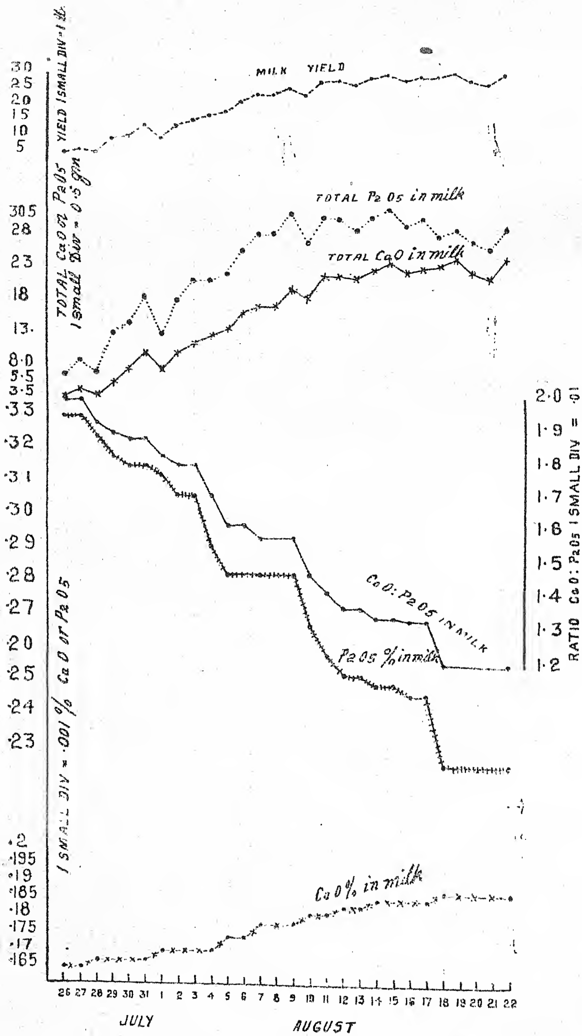

FIG. 1. Filbir—First calver and high yielder; milk yield, etc. from premilk onwards

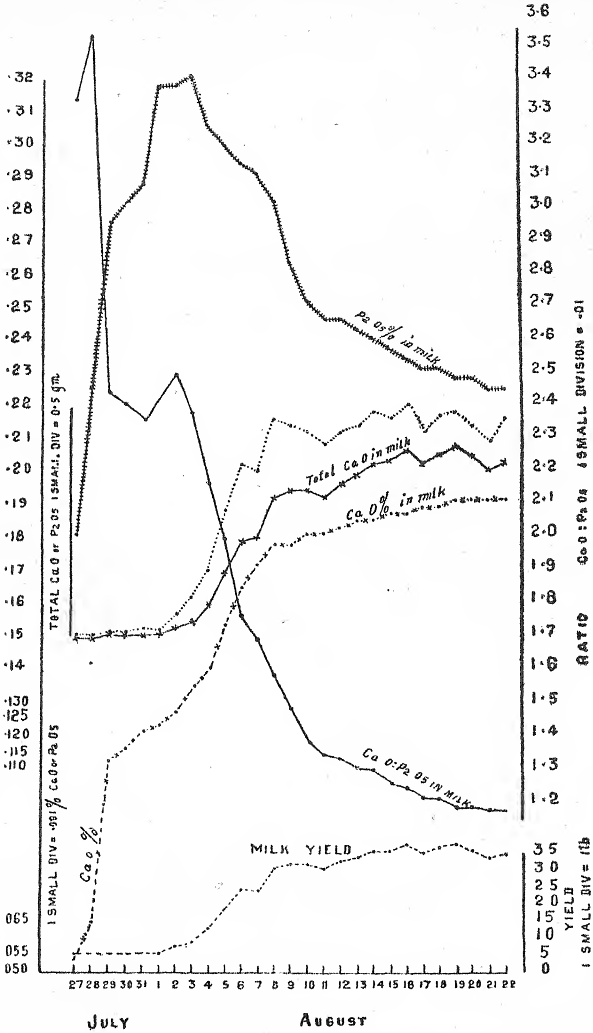

Fig. 2. Narajkati—Second calver and high yielder; milk yield, etc. from premilk onwards

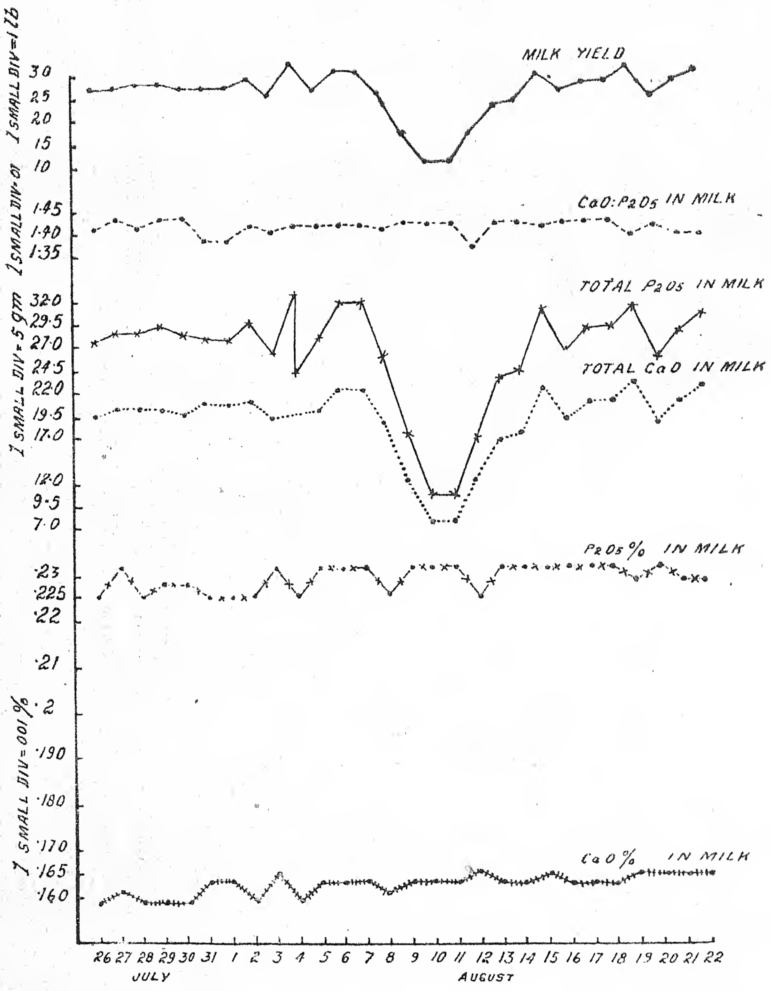

FIG. 3. Nasurki—First calver and high yielder; milk yield, etc. in the first 100 days of lactation

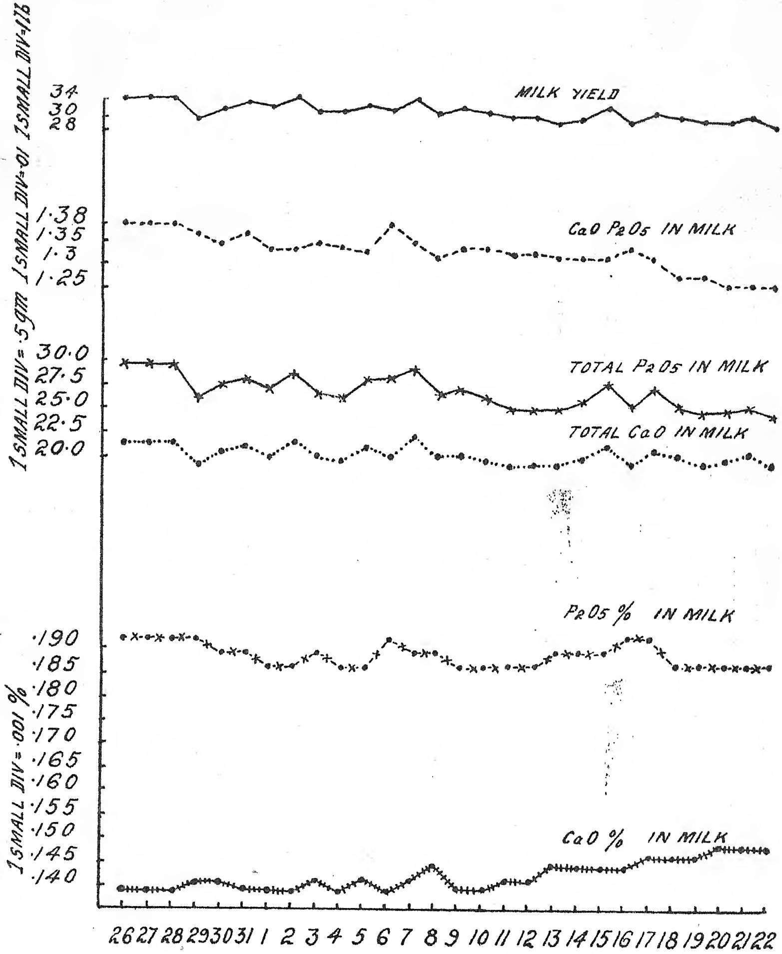

FIG. 4. Tashkam—Second calver and high yielder; milk yield, etc. in the first 100 days of lactation

E

The day-to-day analytical data of some representative cows, as they are classified in Table I, are shown in Figs. 1-12.

Discussion

Premilkers. In the case of 'Felbir' (first calver), the percentage of calcium was low in the premilk and it tended to increase up to the time of calving. The tendency to increase continued for about a week after calving and then it remained constant. On the other hand, the percentage of phosphorus was very high in the premilk and the tendency was to fall immediately after calving; the decrease continued for about a week or two and then it tended to remain stable. The percentage of calcium increased from 0·165 to 0·187 in four weeks time whereas the percentage of phosphorus fell from 0·33 to 0·225. The ratio in the premilk was as high as 1 : 2·0; when the percentage of calcium and phosphorus tended to remain constant after calving, the ratio was 1 : 1·2. The yield of premilk was from 4·5 to 16 pounds per day.

In case of second calver, 'Narajkhati', the examination of milk was started from the very first day of taking out the premilk. In the beginning the yield was half a pound only, but later rose to 8 pounds per day before calving. The percentage of calcium was very low in the beginning and tended to increase as the time of calving approached; it continued to increase gradually for a week or two and tended to remain constant. On the other hand the percentage of phosphorus was high in the beginning, increased and reached the highest two days before calving and then decreased. The decrease continued for about two weeks and then a tendency towards stability appeared. The percentage of calcium increased from 0·054 to 0·191 in four weeks; whereas the percentage of phosphorus rose from 0·186 to 0·321 before calving, then falling gradually reached 0·225. The ratio of calcium to phosphorus

Table VI

Comparative figures for calcium and phosphorus ratio amongst first calvers

Names of cows	Average milk yield per day in lb.	CaO percentage in milk	P_2O_5 percentage in milk	Ratio CaO : P_2O_5.	Stage of lactation
Nashurki	25	0·162	0·228	1 : 1·40	1st 100 days
Tashkam	31	0·144	0·189	1 : 1·32	2nd 100 days
Taroochi	22	0·174	0·237	1 : 1·37	
Folgai	22	0·174	0·237	1 : 1·37	
Lereng	14	0·161	0·234	1 : 1·45	3rd 100 days
Tambutia	12	0·167	0·216	1 : 1·28	
Lakram	10	0·176	0·240	1 : 1·36	

Table VII

Comparative figures for calcium and phosphorus ratio amongst second calvers

Names of cows	Average yield of milk per day in lb.	CaO percentage in milk	P_2O_5 percentage in milk	Ratio CaO : P_2O_5	Stage of lactation
Enarji	38	0·149	0·234	1 : 1·58	1st 100 days
Sakila	28	0·152	0·198	1 : 1·28	
Lanchesari	28	0·159	0·234	1 : 1·4	2nd 100 days
Lakhroochi	26	0·172	0·231	1 : 1·34	
Lakhrajan	22	0·172	0·228	1 : 1·31	
Sarajan	14	0·152	0·210	1 : 1·38	
Rajdhuina	9	0·181	0·255	1 : 1·4	

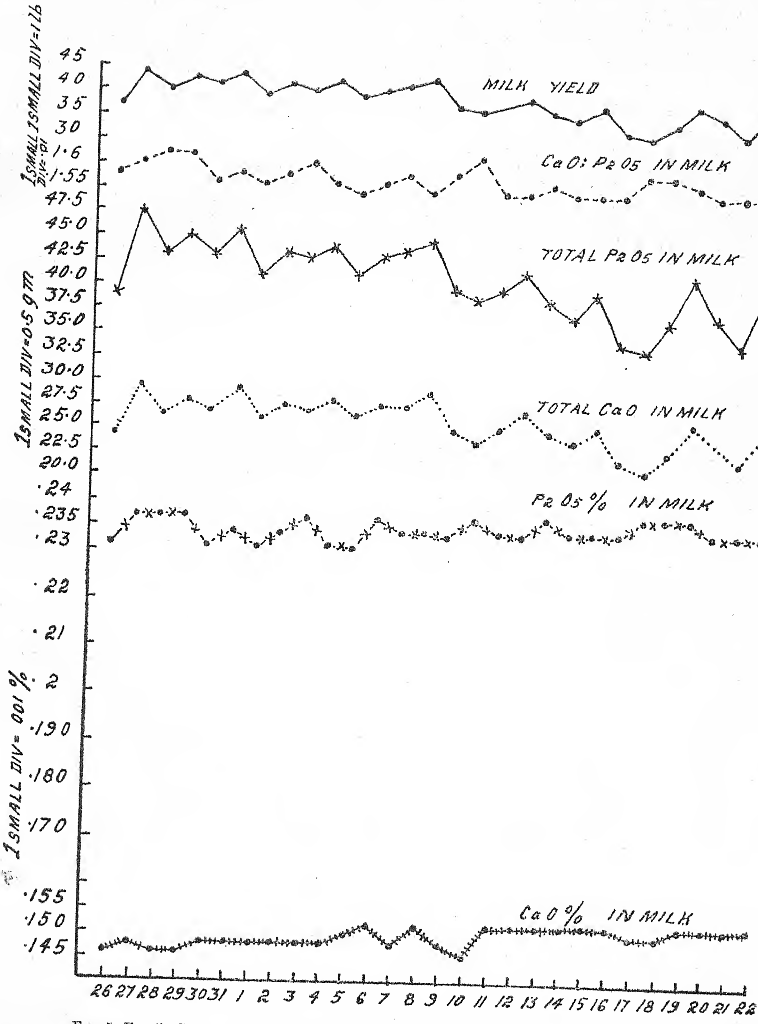

FIG. 5. Enarji—Second calver and high yielder; milk yield, etc. in the first 100 days of lactation

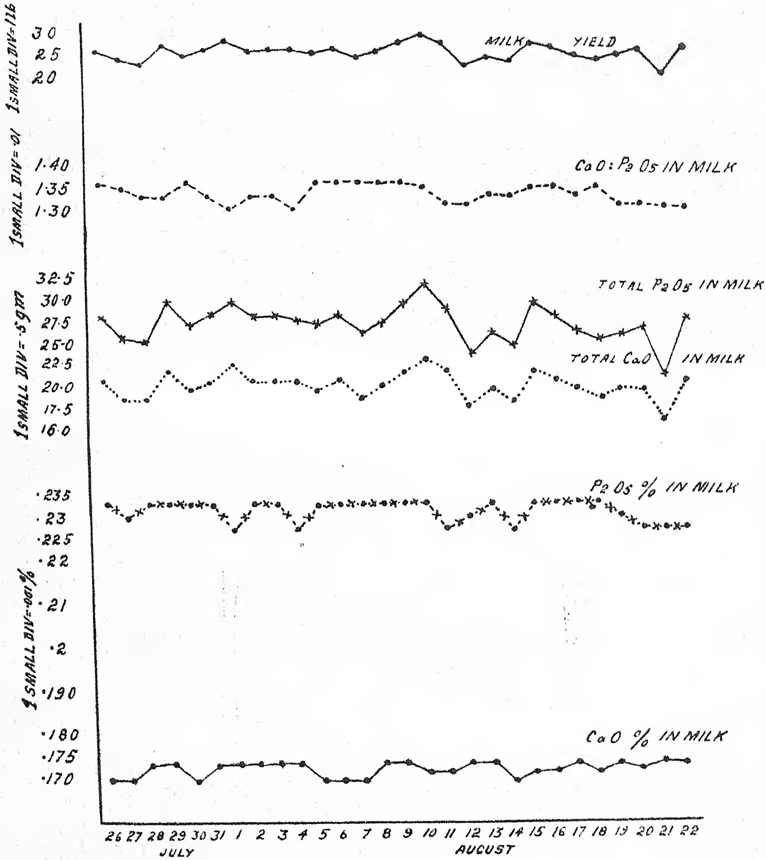

FIG. 6. Lakhroochi—Second calver and high yielder; milk yield, etc. in the second 100 days of lactation

in the premilk was as high as 1 : 3·64 and when the percentage of calcium and phosphorus appeared to remain constant after calving, the ratio was 1 : 1·18.

It was interesting to note that the highest ratio of $CaO : P_2O_5$ was found on the very first or second day of premilk. As 'Felbir' was already giving premilk, the highest ratio was not observed as in the case of 'Narajkhati.' This explained the dissimilarity in the curves of the graphs of the two cows in the first few days. If the part of the curves of Narajkhati's graph for the first few days is not considered, the curves in those of the two premilkers are similar. The high ratio of the salts in 'Narajkhati's' milk indicated more secretive power.

Two high milkers and five medium and low milkers in the first, second and third hundred days of lactation were selected. In 'Tashkam' with a yield of 31 pounds per day the percentage of calcium and phosphorus was low as compared to the others. In the individual cows, there was not much variation in the percentages of calcium and phosphorus in the course of four weeks. In all the first calvers the composition of each cow's milk was different from that of the other. In the third 100 days of lactation, when the yield was low and the cow was completing lactation, the milk was generally richer in mineral contents. It was of interest to note that in 'Taroochi' and 'Folgai' with the same yield (22 pounds) and in the same stage of lactation, the percentage of calcium and the phosphorus was the same.

Four high milkers and three medium and low milkers in the first and second 100 days of lactation were available. In all the cows except diseased ones, there was not much variation in the percentages of calcium and phosphorus in the course of four weeks. The composition of the milk of the different cows varied. In 'Rajudhina' the calcium and phosphorus percentage was rather high for some time when she was suffering from foot-and-mouth disease. It decreased as the cow came to normal health. In some cases, with the decrease in yield in the later part of lactation, the calcium percentage increased.

Four high and four low milkers in the first, second and third 100 days of lactation were selected. 'Rajlari' also was affected with foot-and-mouth disease and this accounted for the high amount of calcium and phosphorus in the milk for some time. The percentage of calcium and phosphorus fell later on when the cow was normal.

A comparison of the data of two cows 'Rajsam' and 'Lakroochi' in the first 100 days and second 100 days of lactation (in Table IX) revealed that:

(1) During the flush (peak) period when the yield reached the maximum, the percentages of calcium and phosphorus were appreciably lower than those at a later period when the yield fell by 12-16 pounds;

(2) The ratio of calcium to phosphorus was practically the same; and

(3) The total amount of calcium and phosphorus output in the milk per day also fell with the yield. There were chances of positive balances being maintained and storage if the rations were not much reduced.

TABLE VIII

Comparative figures for calcium and phosphorus ratio amongst adults

Names of cows	Average yield of milk per day in lb.	CaO percentage in milk	P_2O_5 percentage in milk	Ratio $CaO : P_2O_5$	Stage of lactation
Narbila	37	0·165	0·201	1 : 1·21	1st 100 days
Rajkhati	37	0·159	0·174	1 : 1·09	
Lakholmi	34	0·163	0·189	1 : 1·15	2nd 100 days
Rajsam	28	0·167	0·213	1 : 1·27	
Choreng	16	0·165	0·237	1 : 1·43	3rd 100 days
Rajarama	15	0·157	0·228	1 : 1·45	
Rajsuri	12	0·165	0·225	1 : 1·34	
Rajlari	6	0·185-0·198	0·264-0·273	1 : 1·37-1·25	

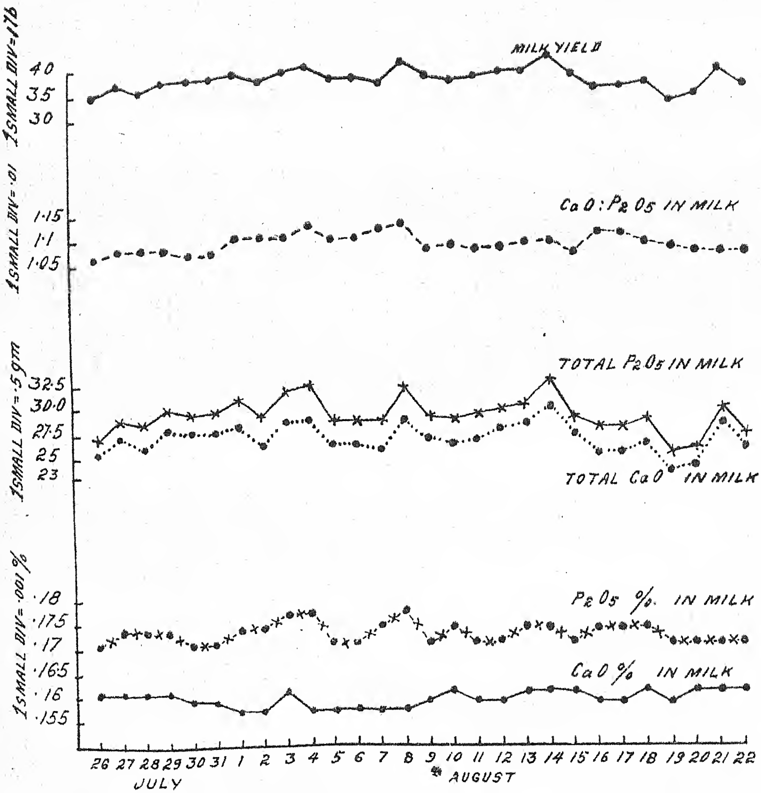

FIG. 7. Rajkhati—Adult and high yielder, milk yield, etc. in the first 100 days of lactation

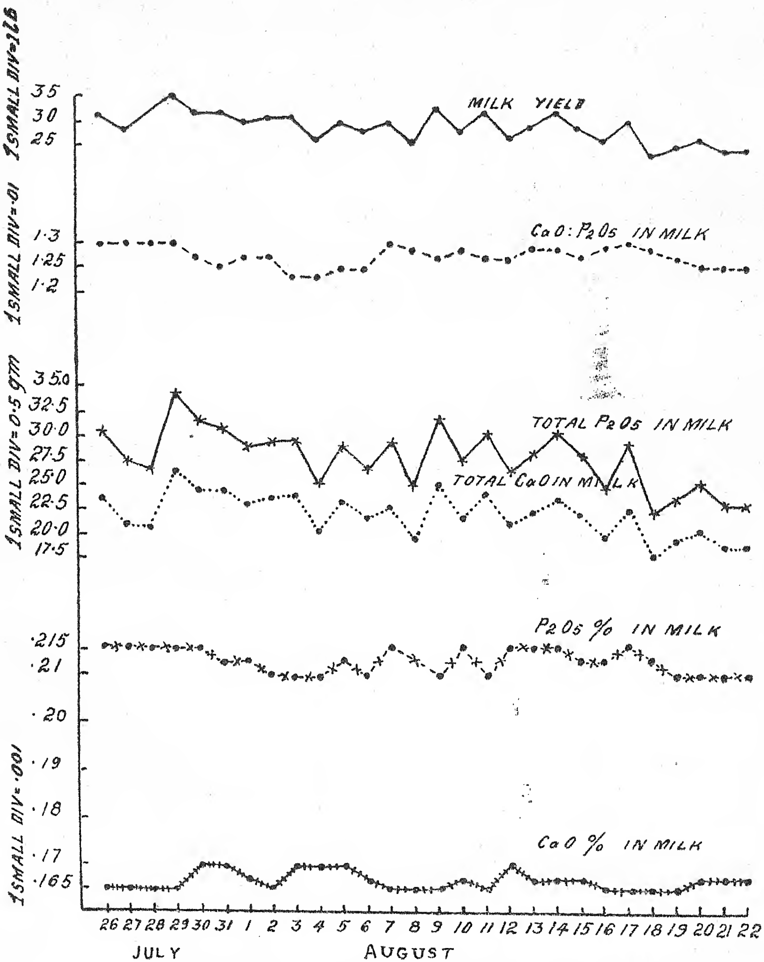

FIG. 8. Rajsam—Adult and low yielder, milk yield, etc. in the second 100 days of lactation

TABLE IX

Comparative data of two cows' milk in different periods of milk yield

Names of cows	Time	Average yield of milk per day in lb.	Average CaO percentage in milk	Average total CaO in milk per day in gm.	Average P_2O_5 percentage	Average total P_2O_5 in milk per day in gm.	Ratio CaO : P_2O_5
Lakhroochi . .	8th week	38	0·158	27	0·209	36	1 : 1·31
	24th to 28th week . . .	26	0·172	21	0·231	28	1 : 1·34
Rajsam	8th week	44	0·153	31	0·193	39	1 : 1·26
	24th to 28th week . . .	28	0·167	21	0·213	27	1 : 1·27

TABLE X

Comparison of the milk yields of American and Indian cows and the percentages of calcium and phosphorus in their milk

Breeds	Yield in lb.	CaO percentage	P_2O_5 percentage
High milkers			
Holstein	35-48	0·179—0·200	0·201—0·204
Holstein	50-58	0·165—0·197	0·196—0·210
Sahiwal	35-45	0·152—0·167	0·174—0·237
Low milkers			
Jersey	18-22	0·164—0·184	0·202—0·210
Holstein	23-32	0·186—0·209	0·214—0·258
Sahiwal	21-22	0·172—0·174	0·218—0·237

In the balance experiments of Hart *et al.* [1922] in the case of animal No. 2 with an average daily yield of 41 pounds, the average of CaO in the fæces and urine was 70·77 per cent and that of P_2O_5 was 66·5 per cent, of the total amount of intake. In all cases they found that the balances were just positive. This showed that for 29 gm. of CaO in milk, 100 gm. of CaO should be given in the feed to keep the balance just positive while for 33 gm. of P_2O_5 in the milk 100 gm. should be given in the feed. When our data are used for calculations on similar lines, positive calcium balances are found in all the cases, phosphorus balances positive in most, but negative in some cases, specially in the case of high-milking cows.

Table XI shows the total intake of calcium and phosphorus in the feed and the average percentage of calcium and phosphorus excreted in the milk from the total intake during the different periods.

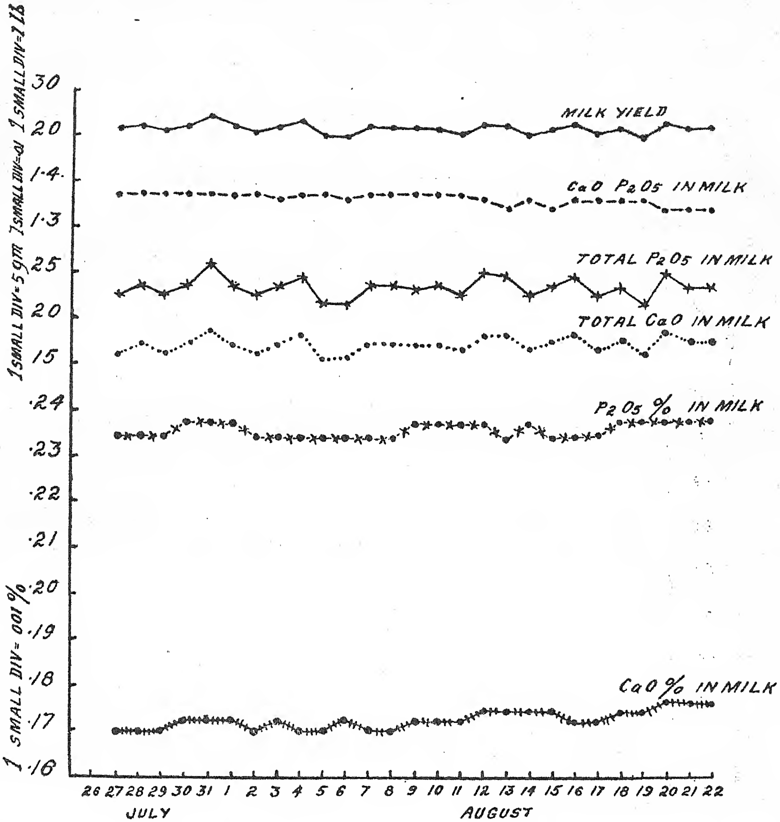

FIG. 9. Taroochin—First calver and low yielder; milk yield, etc. in the second 100 days of lactation

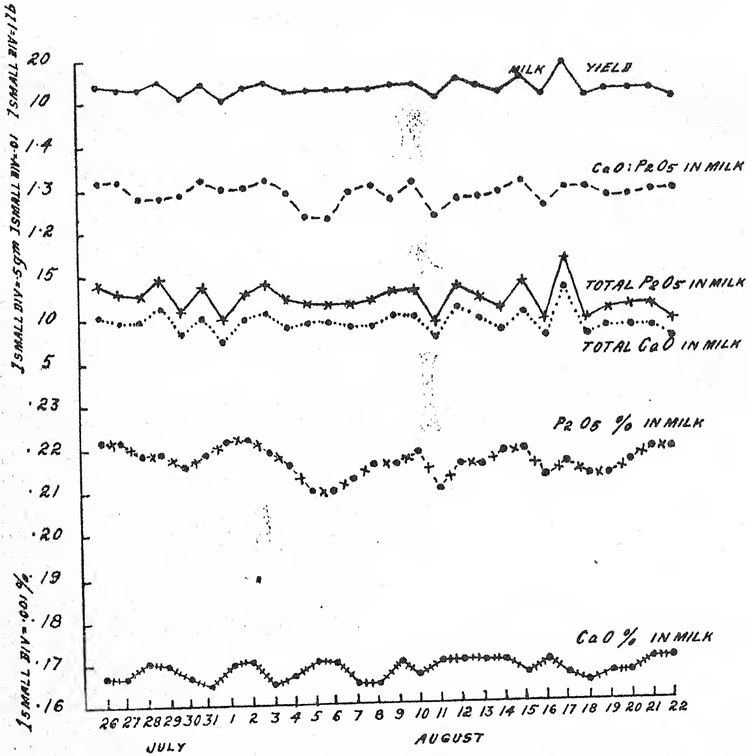

Fig. 10. Tambutia—First calver and low yielder; milk yield, etc. in the third 100 days of lactation

TABLE XI

Total intake of CaO and P_2O_5 in feeds and the percentage excreted in milk

Names of cows	26th to 31st July				1st to 4th August				5th to 10th August			
	Intake of CaO in feed	Average percentage of CaO excreted in milk from the total intake	Intake of P_2O_5 in feed	Average percentage of P_2O_5 excreted in milk from the total intake	Intake of CaO in feed	Average percentage of CaO excreted in milk from the total intake	Intake of P_2O_5 in feed	Average percentage of P_2O_5 excreted in milk from the total intake	Intake of CaO in feed	Average percentage of CaO excreted in milk from the total intake	Intake of P_2O_5 in feed	Average percentage of P_2O_5 excreted in milk from the total intake
High milkers												
1. Felbir	99·57	5·81	57·3	18·6	106·58	9·59	71·7	25·2	107·34	15·66	68·7	*39·05*
2. Narajkhati	97·72	·3	49·52	1·55	104·73	2·23	63·91	7·9	106·61	15·0	62·15	*44·68*
3. Nashurki	105·69	18·8	90·26	31·16	110·85	18·9	96·87	30·0	113·26	14·9	90·13	26·44
4. Tashkam	105·87	19·7	92·35	30·8	111·03	18·28	98·95	30·0	113·38	18·1	91·59	30·0
5. Enarji	105·73	25·42	98·05	*44·67*	112·7	24·1	104·65	*40·95*	114·99	18·33	96·67	*43·0*
6. Sakila	105·69	17·43	90·26	22·29	110·85	18·37	96·87	27·72	113·26	17·93	90·13	29·43
7. Lanchasari	104·03	20·44	84·56	*37·14*	109·19	19·4	91·17	*34·16*	111·66	25·18	85·05	32·39
8. Lakhroochi	104·95	19·44	88·46	31·34	109·19	19·98	97·17	29·62	111·66	18·82	85·05	*33·7*
9. Narbila	107·53	26·66	98·05	*36·18*	112·7	26·79	104·65	*35·18*	114·99	23·41	96·67	*33·45*
10. Rajkhati	107·53	25·0	98·05	29·54	111·77	24·65	100·76	30·62	114·13	23·3	93·4	31·13
11. Lakholmi	106·61	22·42	94·16	30·91	109·93	23·73	92·97	32·67	112·4	23·62	86·86	*35·28*
12. Rajsam	104·95	22·24	88·46	*34·09*	110·11	20·41	95·06	20·67	112·52	19·61	88·32	31·9
Low milkers												
13. Rajsuri	96·65	9·95	53·41	25·26	101·81	9·88	60·01	21·81	104·74	8·85	58·88	20·68
14. Rajrama	99·42	12·09	65·09	26·6	103·66	11·15	67·8	25·28	106·47	10·2	65·43	23·83
15. Choreng	97·57	13·7	57·3	34·06	102·73	18·04	63·9	30·44	100·44	12·22	55·55	32·15
16. Rajudhina	93·88	7·75	41·73	24·55	98·12	5·83	44·44	17·76	101·29	6·44	45·8	20·29
17. Rajjari	93·88	6·82	41·73	22·72	99·05	6·54	48·38	21·45	102·15	5·82	49·07	15·72
18. Sarajan	100·24	11·79	68·99	22·97	105·5	10·34	75·59	19·67	108·2	9·58	71·97	20·67
19. Lakhrajan	101·26	18·63	72·88	34·07	106·42	15·03	79·48	26·78	109·06	15·45	75·24	29·77
20. Tambutia	96·65	10·5	53·41	24·88	101·81	9·21	60·01	20·26	104·74	8·97	58·88	20·38
21. Lerang	95·73	11·39	49·52	32·79	100·89	10·61	56·12	28·45	103·88	10·0	51·61	27·54
22. Lakrami	97·57	9·33	57·3	22·2	102·73	8·71	53·9	19·42	100·45	9·54	55·55	22·24
23. Taroochi	102·18	16·70	76·77	30·63	106·42	16·05	79·48	29·48	109·06	15·18	75·24	30·1
24. Folgai	102·18	17·30	76·77	31·61	106·42	16·79	79·48	31·18	109·06	16·23	75·24	32·1

The italicized figures show negative balances.

TABLE XI—*contd.*

Total intake of CaO and P_2O_5 in feeds and the percentage excreted in milk—contd.

Names of cows	11th to 14th August				15th to 20th August				21st to 22nd August			
	Intake of CaO in feed	Average percentage of CaO excreted in milk from the total intake	Intake of P_2O_5 in feed	Average percentage of P_2O_5 excreted in milk from the total intake	Intake of CaO in feed	Average percentage of CaO excreted in milk from the total intake	Intake of P_2O_5 in feed	Average percentage of P_2O_5 excreted in milk from the total intake	Intake of CaO in feed	Average percentage of CaO excreted in milk from the total intake	Intake of P_2O_5 in feed	Average percentage of P_2O_5 excreted in milk from the total intake
High milkers												
1. Felbir	103·9	20·75	68·63	*43·4*	103·22	22·1	67·27	*42·9*	104·23	21·7	88·77	30·5
2. Narajkhati	105·63	22·45	75·18	*41·9*	104·95	25·9	73·82	*45·0*	105·48	24·15	98·35	30·6
3. Nashurki	108·1	12·2	83·52	22·15	107·42	18·9	82·16	*34·34*	107·6	19·5	104·94	27·53
4. Tashkam	106·36	18·1	81·72	30·9	106·58	18·58	80·35	31·9	106·86	18·2	103·14	23·7
5. Enarji	109·83	23·11	90·07	*43·45*	109·15	21·73	88·7	*47·1*	108·48	21·97	109·73	*33·38*
6. Sakila	108·1	17·1	83·52	28·86	107·42	17·8	82·16	29·5	107·6	15·2	104·94	19·0
7. Lanchasari	106·49	16·75	78·45	*34·34*	105·81	19·19	77·08	*37·89*	105·98	19·3	98·35	30·36
8. Lakhroochi	105·63	18·42	75·19	*34·68*	104·95	19·27	73·82	*34·65*	105·11	18·0	93·56	26·37
9. Narbila	108·96	25·78	86·8	*39·47*	108·28	25·47	85·43	*38·67*	108·48	24·78	109·73	29·06
10. Rajkhati	108·96	25·67	86·8	*34·72*	108·28	23·14	85·43	30·68	108·48	24·12	109·73	25·71
11. Lakholmi	108·1	24·66	83·53	*36·11*	107·42	23·58	82·16	*35·24*	107·6	23·36	104·94	26·93
12. Rajsam	106·36	21·24	81·72	*35·4*	106·58	18·73	80·35	31·73	106·86	17·06	103·14	22·17
Low milkers												
13. Rajsuri	99·58	9·47	52·28	23·26	98·9	8·2	50·91	20·65	98·1	8·45	55·21	20·06
14. Rajrama	100·45	11·24	55·55	29·26	99·77	10·02	54·18	26·91	98·97	9·47	60·03	21·8
15. Choreng	99·58	11·83	52·28	32·23	98·9	11·57	50·9	31·65	98·9	10·01	60·03	23·32
16. Rajudhina	96·13	7·07	39·2	24·21	95·45	6·53	37·83	23·33	95·47	6·34	40·87	21·73
17. Rajlari	96·99	5·0	42·47	16·47	96·31	5·24	41·1	17·22	95·47	3·97	40·87	13·5
18. Sarajan	102·27	9·68	62·09	21·57	100·5	9·5	60·73	21·04	100·73	8·48	69·61	16·7
19. Lakhrajan	102·17	17·48	62·09	37·64	101·49	16·24	60·73	35·55	101·6	16·0	74·4	20·0
20. Tambutia	98·72	9·29	49·01	23·44	98·04	9·41	47·64	24·95	98·1	7·87	55·24	18·0
21. Lereng	98·72	10·84	49·01	31·12	98·04	10·25	47·64	30·26	98·1	10·1	55·24	25·95
22. Lakrami	99·58	8·6	52·28	22·82	98·9	7·76	60·91	20·57	98·1	6·35	55·24	15·34
23. Taroochi	103·9	16·67	68·63	34·37	103·22	16·68	67·27	34·69	103·35	17·0	83·88	28·2
24. Folgai	103·9	17·34	68·63	36·12	103·22	16·26	67·27	34·04	102·48	16·6	79·19	28·88

The italic sized figures show negative balances.

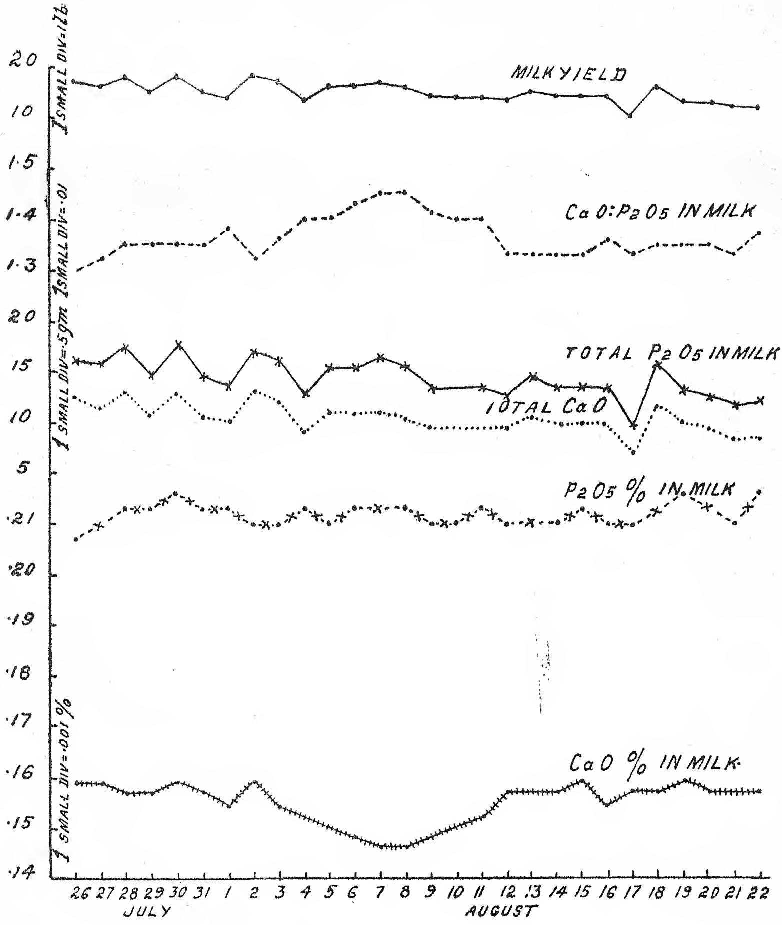

FIG. 11. Sarajan—second calver and low yielder; milk yield, etc. in the second 100 days of lactation

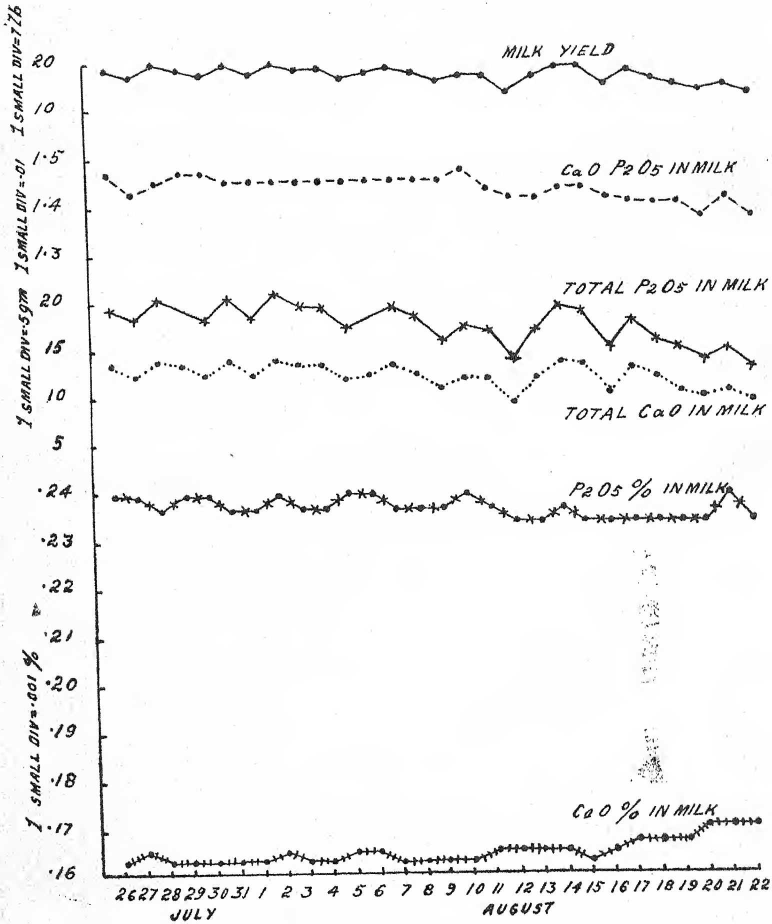

FIG. 12. Choreng—adult and low yielder; milk yield, etc. in the third 100 days of lactation

SUMMARY

The percentages of calcium and phosphorus in the different milkings of a particular day in a cow showed no variation.

There was practically no variation in the percentages of calcium and phosphorus in the course of four weeks except in the premilkers or those cows which reached or passed the peak period.

When the cow was in the peak period the percentages of calcium and phosphorus in the milk decreased and when that period passed, the percentages of these elements in the milk increased.

When the yield was below 10 pounds, in the last days of lactation, the percentage of CaO and P_2O_5 increased.

In the premilk the percentage of phosphorus was very high and that of calcium very low, so much so that the ratio of CaO : P_2O_5 attained a value as high as 1 : 3·64 in the beginning.

The percentage of calcium in the premilk gradually increased and continued to do so till a week or two after calving when the tendency towards stabilization appeared. On the other hand, the percentage of phosphorus increased for some time, reached the maximum before calving and then decreased. It continued to do so for a week or so after calving and then the tendency towards stabilization appeared.

The ratio of calcium to phosphorus was different in individual cows.

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ON THE MORPHOLOGY OF A NEW GENUS OF AMPHISTOMES FROM THE RUMEN OF CATTLE IN THE UNITED PROVINCES

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(Received for publication on 28 October 1944)

(With Plates XII and XIII)

IN the course of investigations by the authors on the systematic survey of the helminth parasites of the domesticated animals at Lucknow under a Scheme sanctioned by the Imperial Council of Agricultural Research, New Delhi, some amphistomes were recovered by the authors, from the rumen of buffaloes and cattle at the slaughterhouses at Lucknow. A few specimens of the amphistomes were also received from Etawah and Bareilly. These, on closer examination, appear to be new to science.

These amphistomes reveal certain points of great systematic value which necessitates the revision of our knowledge of this group of trematodes. While reserving remarks on the classification to a subsequent publication, the authors present here in the following pages a detailed account of the anatomy of this interesting form.

METHODS

The worms were fixed in corrosive sublimate solution but some were flattened by pressure of cover-glass for whole mounts. Several chemical reagents were tried for the study of excretory and the complicated lymphatic systems, but the following method gave the best results. The worms, after washing, were shaken vigorously in a saturated solution of corrosive sublimate in water acidulated by the addition of 3 per cent glacial acetic acid and allowed to remain in this solution for a period of over 18 hours. They were first washed in distilled water for 15 minutes, then in tap water for 15-20 minutes and immediately placed in one per cent aqueous solution of caustic potash for one hour. They were again washed in water, dehydrated, cleared and mounted. The excretory vessels and their branches became black.

For the study of the lymphatic system it was found desirable to keep the specimens in corrosive sublimate for a lesser time, usually six hours. After washing in the usual way they were placed in $\frac{1}{2}$ per cent solution of sodium thiosulphate (hypo) which was allowed to react on the specimens for $\frac{1}{2}$ to 1 hour. The material was then thoroughly washed in water and cleared in glycerine. The lymph vessels took a beautiful yellow tinge and could be clearly traced through the specimens.

Acetic alum carmine and Ehrlich's acid hæmatoxylin gave good results for the whole mounts and sections.

Olveria indica N.G., N.Sp.

The worms are flat and slightly elongated, measuring 6·14-7·07 mm. in length and 2·35-3·35 mm. in greatest breadth. They are widest in the middle and gradually taper towards the anterior end. The posterior end is, however, rounded. There are cuticular papillæ present in the region of the mouth and the genital sucker. The cuticle is inturned both at the mouth and the genital opening and shows cuticular papillæ in these regions. It is produced into small ridges (denticles) at the mouth and is continued into the entire length of the muscular portion of the œsophagus forming its inner lining.

The mouth opening is anterior and terminal and is surrounded by an oral sucker. Arising from the postero-lateral sides of the oral sucker are two pouches, one on each side, which are fused with it.

The acetabulum at the posterior end of the body is well developed and larger than the oral sucker. It is oval in shape and measures 1·3 mm. by 0·73 mm. in size.

The mouth leads into an elongated recurved J-shaped œsophagus which consists of two portions—an anterior long muscular portion which forms the elongated bar and the curve of the letter 'J' and a posterior non-muscular portion which connects the former with the intestinal bifurcation. The non-muscular portion of the œsophagus is surrounded by a large number of unicellular gland cells. The whole of the œsophagus measures 3·6 mm. in length. The cæcal bifurcation is about 1·74 mm. from the anterior end of the body. The cæca are long, coiled slender tubes, making two to three antero-posterior loops in their course, and, terminating blindly near the ovary, in front of the posterior sucker.

The excretory pore is situated dorsally at the posterior end of the body immediately in front of the posterior sucker and a little behind the opening of the Laurer's canal. The excretory bladder is fairly large and more or less rounded in shape. It is placed dorsally slightly overlapping the posterior sucker. The two longitudinal excretory ducts arise from the bladder, one on each side and, run forward towards the anterior end, terminating at the level of the oral pouches. During its course about the middle of the body, each longitudinal duct gives out internally one branch which runs towards the median line and meets the fellow of the other side at about the level of the bend of the J-shaped œsophagus and thus gives an H-shaped appearance to the excretory system which is essentially a *Paramphistomum* pattern. A small branch from the point of junction of the two transverse ducts runs anteriorly to the œsophagus. Other small branches are also given off from the longitudinal ducts and they are mainly distributed over the intestinal cæcum of each side and thus form a complete network of excretory tubules round them. It may be pointed out here that it is the first record of a network of excretory tubules surrounding the intestinal cæca in the Amphistomes and is represented in the accompanying microphotograph (Plate XII, fig. 3). Still smaller branches and their sub-branches run to every part of the body and the tubules anastomose with each other.

The lymphatic system consists of two longitudinal vessels, one on each side of the body, running slightly internal to the intestinal cæca. They send out branches in all directions, dorsally, ventrally, laterally and mesially. The main longitudinal vessels as studied in the whole mounts and confirmed by a study of the serial sections are as follows: At the posterior end of the body each longitudinal vessel breaks up into three branches which divide and extend into the tissues of the posterior sucker. Immediately in front of the sucker they form a lymphatic plexus on each side. Two branches are supplied to the excretory bladder from each side, which get distributed round the bladder in small ampulla-like vessels. Anteriorly each longitudinal vessel gives out one branch to the testis of its side and a few to the intestinal cæca. The ovary gets its supply from one of the branches supplying the testis. More anteriorly each longitudinal duct gives out one branch internally which divides and supplies the genital sucker and the œsophagus, and, further forwards divides and sub-divides in the substance of the oral pouch and oral sucker. Just before reaching the oral sucker the longitudinal ducts form another but smaller plexus on each side. The lateral branches and their sub-branches have been observed to fuse with each other and thus form a close network of lymph tubules all over the body.

Female reproductive organs. The ovary is rounded in shape, measuring 0·4-0·43 mm. in diameter, and is situated at a distance of 1·1-1·13 mm. from the posterior end of the body, slightly to the left of the median line near the termination of the cæcum of the side. The oviduct arises from the middle of the mesial side of the ovary. Immediately afterwards it receives the common vitelline duct and the Laurer's canal and forms the öotype. The point of

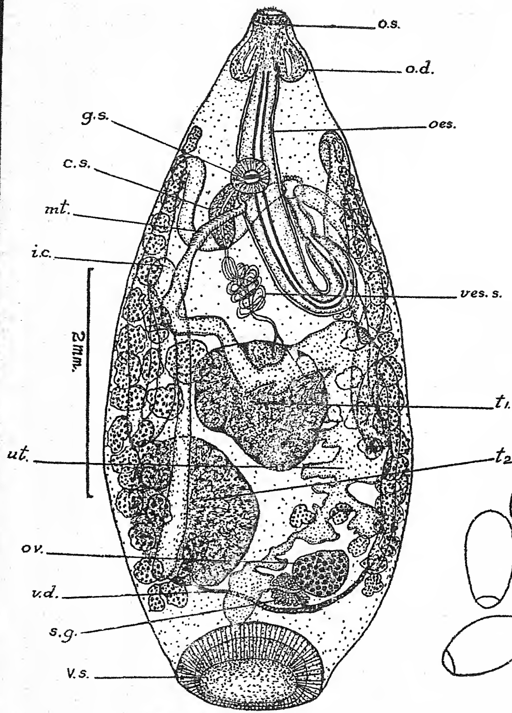

FIG. 1. *Olveria indica*, general anatomy. Ventral view.

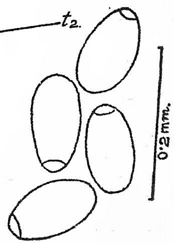

FIG. 2. Eggs

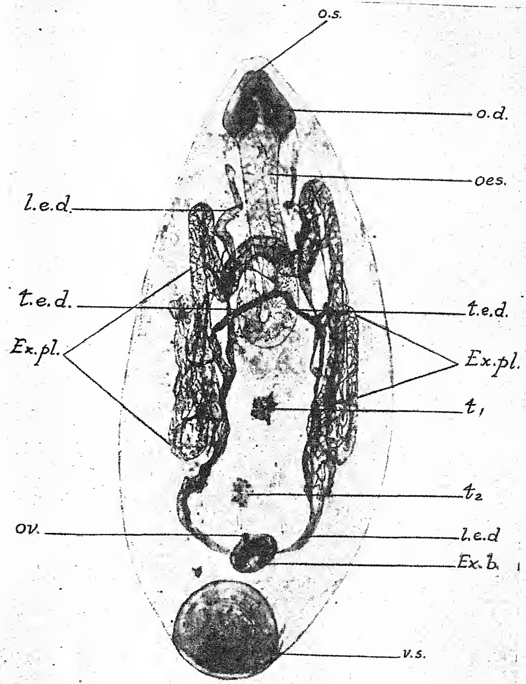

FIG. 3. Microphotograph of the excretory system.

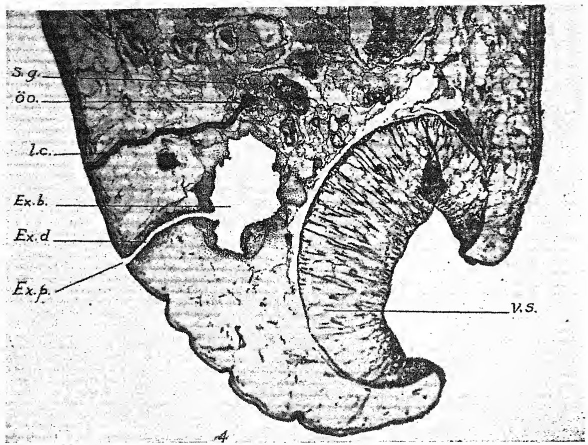

FIG. 1. Microphotograph of a portion of a longitudinal vertical section showing the Laurers canal (*l.c.*) and the excretory duct (*ex.d.*)

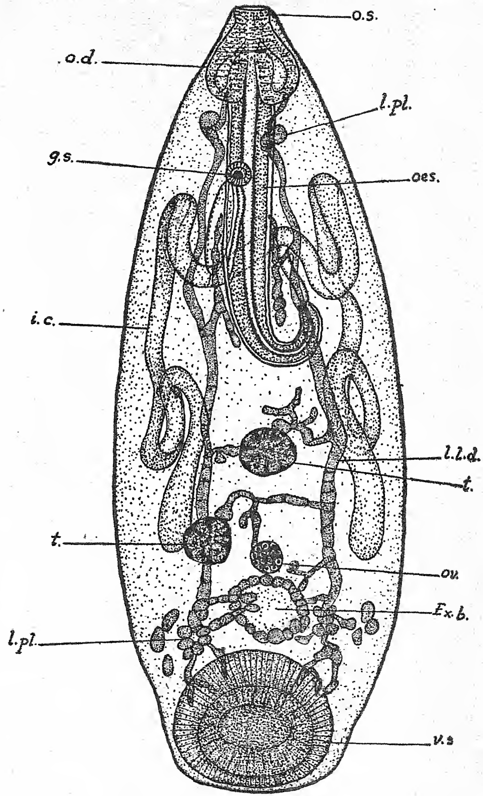

FIG. 2. The lymphatic system and ampullae (vitellaria and uterus omitted)—a reconstruction

junction of the three ducts is further marked by the presence of compact shell-gland cells. The uterus arises from the ötype and runs towards the anterior end of body making a few coils which are full of eggs. The course of the uterus anteriorly is S-shaped, it crosses over from one side of the body to the other and again to the same side as in *Gastrothylax*. Near its termination it becomes slightly muscular and forms the metraterm. The genital pore is situated at a distance of 1·42 mm. from the anterior end of the body and is surrounded by a well-developed genital sucker. The eggs are oval and operculated, measuring 0·068-0·085 mm. by 0·075-0·119 mm. in size.

The vitelline glands are well developed and consist of large follicles situated laterally on either side of the body, extending from a little in front of the genital sucker to a little in front of the posterior sucker. The transverse vitelline duct of each side leaves the glands in front of the acetabulum and runs inwards to meet each other behind the ovary. From this point a common vitelline duct takes its origin and runs forwards to the ötype.

Male Reproductive Organs. There are two testes situated slightly diagonally one behind the other in the posterior half of the body, a little in front of the ovary. They are more or less rounded with slightly lobed margins. The anterior testis lies at a distance of 2·95 mm. from the anterior end of the body and measures 1·2 mm. by 0·95 mm. in size. The posterior testis lies 0·17 mm. behind the anterior testis and measures 1·33 mm. by 1·14 mm. The vasa deferentia from the two testes unite together a little in front of the anterior testis and form a much coiled tube, the vesicula seminalis which leads into a cirrus 0·57 mm. by 0·22 mm. in size. The cirrus is enclosed within a thin-walled cirrus sac, containing numerous prostate gland cells, and open at the genital pore beside the opening of the metraterm.

To sum up the present form, *Olveria*, is characterized thus :—

1. Body pyriform without spines, except in the region of the oral sucker and the genital sucker.
2. Mouth opening terminal; cuticle inside mouth thrown into denticles; two oral pouches present, fused with the oral sucker.
3. Pharynx absent. Oesophagus long and J-shaped, consisting of two portions and is lined with cuticle.
4. Cæca long, forming antero-posterior loops.
5. Excretory system simple, H-shaped; excretory pore at posterior end behind opening of Laurer's canal.
6. Lymphatic system consists of only one pair of longitudinal lymph vessels.
7. Testes two, placed obliquely in posterior half of body. Vesicula seminalis much coiled; cirrus-sac present.
8. Ovary post-testicular, a little to left of median line. Vitellaria in distinct follicles, on the lateral sides of body.
9. A genital sucker present.
10. Eggs operculated and oval in shape.

Discussion

From the foregoing description it appears that the genus *Olveria* shows close affinities with the members of the family Cladorchidæ in the presence of oral diverticula. It further resembles the subfamily Cladorchinæ in the nature of the intestinal cæca, the relative position of the ovary and the testes, the nature and position of the yolk glands and the presence of a genital sucker. It, however, differs from it in the presence of only one pair of longitudinal lymphatic ducts, in the nature of the uterine coils and in the division of œsophagus into an anterior muscular and a posterior glandular portion. In the presence of only one pair of longitudinal lymphatic ducts and the nature of the excretory system, it resembles the members of the family Paramphistomidæ but differs from them in the peculiar division of the œsophagus into two parts and in the presence of oral diverticula and antero-posterior loops in the intestine. In the disposition of the uterine coils it is associated with Gastrothylacidæ from which it differs in many important features including the absence of the ventral pouch and in the peculiar division of the œsophagus into an anterior muscular and a posterior glandular portion. In the division of the œsophagus into two parts it is unique amongst the Amphistomes and it, therefore, cannot be placed under any of the known genera. It, however, combines in itself, as already indicated, the characters of several families of Amphistomes. Further investigations may lead to the creation of a new subfamily for these forms.

Summary

A new genus and species of an Amphistome parasite recovered from the rumen of cattle and buffalo from U. P. has been described in detail. The characteristics of this form are: the presence of oral pouches fused with the oral sucker, the shape and structure of the œsophagus, the presence of antero-posterior loops in the intestinal cæca, the presence of a genital sucker, H-shaped excretory vessel and one pair of longitudinal lymphatic ducts. The presence of the intestinal cæca has been demonstrated for the first time in this genus and is likely to be present in other genera of Amphistomes as well.

Methods for the study of excretory and lymphatic systems have been described.

ACKNOWLEDGEMENTS

Our grateful acknowledgements are due to the authorities of the Imperial Council of Agricultural Research for the facilities offered to carry out the investigations at Lucknow. The genus is being named after Sir Arthur Olver whose unfailing interest in the improvement of cattle in India had made possible the sanction of the present Scheme at Lucknow.

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LETTERING ON THE FIGURES PLATES

c.s., cirrus sac
ex.b., excretory bladder
ex.d., excretory duct
ex.p., excretory pore
g.s., genital sucker
i.c., intestinal cæcum
l.c., Laurer's Canal
l.e.d., longitudinal excretory duct
l.l.d., longitudinal lymphatic duct
l.pl., lymph plexus
mt., metraterm
œs., œsophagus
o.p., oral pouch
öo., öotype
o.s., oral sucker
ov., ovary
p.s., posterior sucker
s.g., shell gland
t1, anterior testis
t2, posterior testis
t.e.d., transverse excretory duct
vit., vitellaria
ut., uterus
v.d., vitelline duct
v.s., acetabulum
ves.s., vesicula seminalis

RESULTS OF A SURVEY ON THE NATURE AND INCIDENCE OF HELMINTH INFECTION IN CATTLE, GOATS AND SHEEP IN THE CENTRAL PROVINCES AND BERAR AND CENTRAL INDIA

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(Received for publication on 25 June 1945)

A SCHEME of research at Nagpur was sanctioned by the Imperial Council of Agricultural Research in May, 1940. The work in connection with the scheme commenced in September, 1940 and closed on 31 March 1943. The programme of work under this scheme was restricted to the investigation of (i) the nature of helminth infection and (ii) the incidence of infection in goats, sheep and cattle. During the continuance of this scheme, parasites sent to us from the Veterinary Departments of Baroda, Bombay and the Central Provinces and Berar were identified; samples of fæces were examined and a few parasites from poultry were also collected and identified. This paper embodies the results of the survey with reference only to goats, sheep and cattle. From its very nature, it is evident that the work is mainly statistical.

MATERIAL AND METHOD

For the purpose of this survey, collections were made at Nagpur, Jubbulpore, Raipur, Hoshangabad, Narsingpur, all in the Central Provinces, at Amraoti in Berar, and at Indore and Mhow in Central India. Viscera of animals was obtained from the municipal slaughterhouses and thorough examinations for parasites were made. A fairly large number of animals of each kind have been examined and the examinations carried out in different seasons. These factors, in the author's opinion, have made the results reliable and instructive. The number of animals examined at each place is given in Table I.

TABLE I

Number of animals examined at each place

Place	Goat	Sheep	Cattle
Nagpur	101	107	33
Jubbulpore	6	13	3
Raipur	6	6	8
Hoshangabad	2	...	1
Narsingpur	4	...	2 (including one buffalo)
Amraoti	3	4	3
Indore	12	12	...
Mhow	...	...	14

For summarizing the results of the survey in this paper the author has taken the data obtained from Nagpur, Jubbulpore, Amraoti and Central India.

NATURE OF INFECTION

The parasites obtained from goat, sheep and cattle are given in Table II.

TABLE II

Parasites obtained from goat, sheep and cattle

Parasites from goat, sheep and cattle	Nagpur (Central Provinces)		Jubbulpore (Central Provinces)		Amraoti (Berar)		Mhow and Indore (Central India)*	
	+ if present	Host	+ if present	Host	+ if present	Host	+ if present	Host
Trematodes								
1. *Cotylophoron cotylophorum*	+	Goat, sheep, cattle	+	Goat, sheep, cattle			+	Cattle
2. *C. ovatum*	+	Goat, sheep, cattle					+	Sheep, cattle
3. *Gastrothylax crumenifer*	+	Goat, sheep, cattle	+	Goat, sheep, cattle			+	Cattle
4. *Paramphistomum cervi*	+	Goat, sheep, cattle	+	Sheep, cattle			+	Cattle
5. *Fasciola gigantica*	+	Goat, sheep, cattle	+	Goat, sheep, cattle			+	Cattle
6. *Fasciola hepatica*	+	Goat, sheep, cattle	+	Goat, sheep	+	Sheep	+	Cattle
7. *Schistosoma spindalis*	+	Goat, sheep, cattle	+	Goat, sheep, cattle				
8. *S. indicum*	+	Goat, sheep, cattle	+	Sheep, cattle	+	Sheep, cattle	+	Goat, sheep, cattle
9. *S. bovis*	+	Goat, sheep, cattle	+	Goat, sheep	+	Goat, sheep	+	Cattle
10. *S. haematobium*	+	Goat, sheep, cattle						
Cestodes								
1. *Cysticercus ovis*	+	Goat, sheep	+	Sheep	+	Sheep, cattle		
2. *Stilesia vittata*	+	Goat, sheep	+	Goat, sheep	+	Goat	+	Goat, cattle, sheep
3. *S. globipunctata*	+	Goat, sheep	+	Goat, sheep	+	Sheep	+	Goat, cattle, sheep
4. *S. hepatica*	+	Goat, sheep	+	Goat, sheep	+	Goat	+	Goat, cattle, sheep
5. *Avitellina lahorea*	+	Goat, sheep	+	Goat, sheep				
6. *A. centripunctata*	+	Goat, sheep	+	Sheep			+	Goat, sheep
7. *A. woodlandi*	+	Goat, sheep						
8. *A. sudanea*	+	Goat, sheep					+	Sheep
9. *A. goughi*	+	Goat, sheep	+	Sheep				
10. *Moniezia expansa*	+	Goat, sheep, cattle						
11. *M. benedini*	+	Goat, sheep, cattle	+	Sheep				
12. *Echinococcus granuloses*	+	Goat, sheep, cattle	+	Goat, sheep			+	Cattle
13. *Cysticercus bovis*	+	Cattle						
14. *Cysticercus tenuicollis*	+	Goat, sheep, cattle	+	Goat, sheep			+	Goat, sheep
Nematoda								
1. *Trichocephalus ovis*	+	Goat, sheep, cattle	+	Goat			+	Goat, sheep, cattle
2. *Hæmonchus contortus*	+	Goat, sheep, cattle	+	Goat, sheep	+	Goat, sheep	+	Goat, sheep, cattle
3. *H. similis*	+	Cattle						
4. *Bunostomum trigonocephalum**	+	Goat, sheep	+	Goat, sheep	+	Goat	+	Goat, sheep
5. *Bunostomum phlebotomum**	+	Sheep, goat					+	Sheep
6. *B. bovis*	+	Cattle	+	Cattle			+	Sheep
7. *Oesophagostomum columbianum*	+	Goat, sheep, cattle	+	Goat, sheep, cattle	+	Sheep	+	Sheep
8. *O. radiatum*	+	Goat, sheep, cattle	+	Goat, sheep	+	Goat, sheep	+	Sheep, cattle
9. *O. venulosum*	+	Goat, sheep, cattle	+	Sheep			+	Sheep, cattle
10. *Gaigeria pachycelis*	+	Goat, sheep	+	Goat, sheep			+	Goat, sheep
11. *Gongylonema pulchrum*	+	Goat, sheep, cattle	+	Cattle			+	Cattle
12. *G. verrucosum*	+	Goat, sheep, cattle					+	Cattle
13. *Setaria digitata*	+	Cattle	+	Sheep, cattle			+	Cattle
14. *S. labiato-papillosa*	+	Cattle					+	Cattle
15. *Mecistocirrus digitatus*	+	Cattle	+	Cattle			+	Cattle
16. *Cooperia curticei*	+	Goat, sheep	+	Goat, sheep				
17. *Ostertagia ostertagi*	+	Goat, sheep, cattle	+	Goat, sheep	+	Goat		
18. *O. circumcincta*	+	Goat, sheep						
19. *Trichostrongylus colubriformis*	+	Goat, sheep	+	Goat				
20. *Strongyloides papillosum*	+	Goat, sheep					+	Goat, sheep

*At Mhow only cattle were examined. At Indore only goats and sheep were examined.

It is evident from Table II that most of the common parasites found in India occur also in the C. P. nd Berar and Central India.

INCIDENCE OF INFECTION

I have already emphasized that the purpose of his survey was to obtain mainly statistical data on the relative degree of infection by the various parasites. Tables III, IV and V give the data obtained.

Table III

Relative degree of infection by various parasites—Host : Goat

Host : Goat	Nagpur					Jubbulpore					Amraoti					Mhow and Indore				
			Degree of Infection					Degree of Infection					Degree of Infection					Degree of Infection		
Parasite	No. of animals examined.	Percentage of Infected animals	Minimum	Maximum	Average	No. of animals examined	Percentage of Infected animals	Minimum	Maximum	Average	No. of animals examined	Percentage of Infected animals	Minimum	Maximum	Average	No. of animals examined	Percentage of Infected animals	Minimum	Maximum	Average
Trematodes																				
Cotylophoron cotylophorum	101	43	3	2025	146	6	33	2	3	3										
C. ovatum	101	9	2	67	16															
Gastrothylax crumenifer	101	16	2	320	56	6	16	..	..	1										
Paramphistomum cervi	101	5	5	40	34															
Fasciola gigantica	101	17	1	40	6	6	16	..	..	1										
Fasciola hepatica	101	8	2	33	14	6	16	..	..	1										
Schistosoma spindalis	101	8	1	50	10	6	16	..	..	1										
S. indicum	101	8	1	12	5											12	16	2	7	4
S. bovis	101	4	2	33	11	6	16	..	..	1	3	66	2	3	2·5					
S. haematobium	101	2	2	5	5															
Cestodes																				
Cysticercus ovis	101	37	1	620	3															
Stilesia vittata	101	28	2	112	35	6	66	13	42	39	3	100	2	3	2·6	12	21	2	90	32
S. globipunctata	101	15	3	450	49	6	16	..	..	48						12	16	3	12	8
S. hepatica	101	14	3	86	23	6	16	..	..	77	3	66	2	3	2·5	12	16	2	9	6
Avitellina lahorea	101	11	2	50	14	6	16	..	..	2										
A. centripunctata	101	12	3	68	11											12	8	90	90	90
A. woodlandi	101	2	4	11	7															
A. sudanea	101	10	1	50	11															
A. goughi	101	2	2	5	3·5															
Moniezia expansa	101	5	2	9	6															
M. benedini	101	3	1	4	3															
Echinococcus granulosus	101	4	1	17	6	6	16	..	..	3										
Cysticercus tenuicollis	101	2	1	3	2	6	16	..	..	3						12	32	1	3	2
Cysticercus cellulosae																12	8	1	1	1
Nematodes																				
Trichocephalus ovis	101	45	1	40	13	6	100	4	111	47						12	81	1	3	38
Haemonchus contortus	101	57	2	600	45	6	100	2	26	15	3	100	40	70	57	12	73	4	400	63
Bunostomum trigonocephalum	101	45	1	105	22	6	33	2	3	3	3	66	20	25	22·5	12	24	4	9	7
Oesophagostomum columbianum	101	32	4	200	28	6	33	8	13	10										
Oe. radiatum	101	19	2	100	24	6	16	..	..	2	3	66	20	30	25					
Bunostomum phlebotomum	101	18	5	68	19															
Gaigeria pachyscelis	101	22	4	80	15	6	33	3	4	3						12	8	1	1	1
Gongylonema pulchrum	101	5	1	18	10															
G. verrucosum	101	7	2	15	4															
Cooperia curticei	101	11	3	40	3	6	16	..	..	2										
Ostertagia ostertagi	101	6	6	150	18	6	33	3	7	5	3·66	66	14	14	14					
O. circumcincta	101	1	8	8	8															
Trichostrongylus colubriformis	101	4	8	260	61	6	16	..	..	1										
Strongyloides papillosum	101	2	2	76	39											12	8	700	700	700
Oesophagostomum venulosum	101	5	3	120	21															

TABLE IV

Relative degree of infection by various parasites—Host: Sheep

Host: Sheep	Nagpur					Jubbulpore				
			Degree of Infection					Degree of Infection		
Parasite	No. of animals examined	Percentage of Infected animals	Minimum	Maximum	Average	No. of animals examined	Percentage of Infected animals	Minimum	Maximum	Average
Trematodes										
Cotylophoron cotylophorum	107	51	3	984	96	8	50	50	400	229
C. ovatum	107	2	3	5	4					
Gastrothylax crumenifer	107	20	3	696	91	8	38	40	260	170
Paramphistomum cervi	107	3	12	12	4	8	13	..	..	60
Fasciola gigantica	107	3	4	19	8	5	20	..	..	3
F. hepatica	107	21	1	42	4	5	20	..	..	2
Schistosoma spindalis	107	6	3	45	9	8	25	4	16	10
S. indicum	107	19	1	24	5	5	40	1	3	2
S. bovis	107	5	2	7	5	5	20	..	..	2
S. haematobium	107	3	1	4	3					
Cestodes										
Cysticercus ovis	107	34	1	7	3	5	20	..	..	2
Stilesia vittata	107	19	1	2000	26	5	60	2	32	21
S. globipunctata	107	17	1	400	49	5	20	..	..	29
S. hepatica	107	22	1	105	34	5	20	..	..	112
Avitellina lahorea	107	9	3	210	12	8	25	3	16	10
A. centripunctata	107	7	2	20	13	5	20	..	..	7
A. woodlandi	107	2	8	50	20					
A. sudanea	107	5	3	8	4					
A. gouphi	107	2	13	15	14	5	20	..	..	5
Moniezia expansa	107	3	1	6	5					
M. benedini	107	2	1	20	12	8	13	..	..	4
Echinococcus granulosus	107	7	1	6	2	8	13	..	..	2
Cysticercus tenuicollis	107	3	1	5	2	8	13	..	..	2
Nematodes										
Trichocephalus ovis	107	75	1	45	8	5	100	2	23	13
Haemonchus contortus	107	74	2	500	55	5	100	28	270	150
Bunostomum trigonocephalum	107	32	2	150	28	5	40	4	8	6
Oesophagostomum columbianum	107	33	1	90	21	8	50	7	60	24
Oe. radiatum	107	38	1	258	37	8	25	10	18	14
Bunostomum phlebetomum	107	11	3	80	19					
Gaigeria pachycelis	107	10	3	15	9	5	20	..	..	4
Gongylonema pulchrum	107	4	2	6	4					
G. verrucosum	107	3	9	7	10					
Setaria digitata						8	13	..	..	4
Cooperia curticei	107	5	3	25	8	5	20	..	..	3
Ostertagia ostertagi	107	1	62	62	62	5	40	2	9	5
Trichostongylus colubriformis	107	5	15	270	87	5	20	..	..	9
Strongyloides papillosum						5	20	..	..	31
Oesophagostomum venulosum	107	6	12	47	24	5	40	7	18	12

Host: Sheep	Amraoti					Mhow and Indore				
			Degree of Infection					Degree of Infection		
Parasite	No. of animals examined	Percentage of Infected animals	Minimum	Maximum	Average	No. of animals examined.	Percentage of Infected animals	Minimum	Maximum	Average
Trematodes										
Cotylophoron cotylophorum										
C. ovatum						12	16	2	4	82
Gastrothylax crumenifer										
Paramphistomum cervi										
Fasciola gigantica										
F. hepatica	4	25	..	..	1					
Schistosoma spindalis										
S. indicum	4	75	2	13	8	12	16	1	1	1
S. bovis	4	25	..	..	4					
S. haematobium										
Cestodes										
Cysticercus ovis	4	25	..	..	2					
Stilesia vittata						12	8	..	..	8
S. globipunctata	4	75	1	9	3					
S. hepatica						12	24	2	8	5
Avitellina lahorea										
A. centripunctata						12	8	..	..	3
A. woodlandi										
A. sudanea						12	8	..	..	8
A. gouphi										
Moniezia expansa										
M. benedini										
Echinococcus granulosus										
Cysticercus tenuicollis						12	16	2	4	3
Nematodes										
Trichocephalus ovis						12	89	1	35	8
Haemonchus contortus	4	75	40	100	67	12	73	1	200	42
Bunostomum trigonocephalum						12	57	2	25	14
Oesophagostomum columbianum	4	75	10	80	34	12	16	3	8	6
Oe. radiatum	4	25	150	150	150	12	8	..	..	2
Bunostomum phlebetomum						12	16	20	25	22
Gaigeria pachycelis										
Gongylonema pulchrum						12	24	4	11	7
G. verrucosum										
Setaria digitata										
Cooperia curticei										
Ostertagia ostertagi										
Trichostongylus colubriformis										
Strongyloides papillosum										
Oesophagostomum venulosum										

TABLE V

Relative degree of infection by various parasites—Host: Cattle

Host: Cattle	Nagpur					Jubbulpore					Amraoti					Mhow and Indore*				
			Degree of Infection					Degree of Infection					Degree of Infection					Degree of Infection		
Parasites	No. of animals examined	Percentage of Infected animals	Minimum	Maximum	Average	No. of animals examined	Percentage of Infected animals	Minimum	Maximum	Average	No. of animals examined	Percentage of Infected animals	Minimum	Maximum	Average	No. of animals examined	Percentage of Infected animals	Minimum	Maximum	Average
Trematodes																				
Cotylophoron cotylophorum	33	72	66	4000	899	2	50	..	..	6						10 4	30 75	15 14	50 40	33 32
Gastrothylax crumenifer	33	54	5	1500	479	2	100	128	3400	1764						Not in cattle 4	25	..	..	11
Paramphistomum cervi	33	21	4	600	282	1	100	..	..	15						10 4	30 50	7 7	250 45	110 26
Fasciola gigantica	33	9	2	9	3	1	100	..	..	23						10 4	10 50	.. 8	.. 12	4– 10
Fasciola hepatica	33	9	1	9	5											10 4	30 50	2 7	90 15	32 11
Schistosoma spindalis	33	15	20	250	130	1	100	..	..	16						10 Not found in buffalo	10	..	..	9
Schistosoma indicum	33	51	8	900	145	2	50	..	..	42	8	67	3	60	35	10 Not found in buffalo	10	..	..	40
Schistosoma bovis	33	12	1	350	170											10 Not found in buffalo	10	..	..	60
Schistosoma haematobium	33	12	20	300	159											10 Not found in buffalo	10	..	..	3
Fischœderius elongatus																Not found in cattle 4	25	..	..	32
Cestoda																				
Cysticercus ovis											13	16	2	3	3					
Stilesia vittata																10 Not found in buffalo	10	..	..	6
Moniezia expansa	33	6	2	6	4															
M. benedini	33	4	..	..	6															
Echinococcus granulosus	33	12	1	9	6											10 4	30 25	5 ..	20 ..	11 4
Cysticercus bovis	33	3	..	..	20															
Cysticercus tenuicollis	33	3	..	..	20											10 4	10 25	.. ..	.. ..	2 2
Nematodes																				
Trichocephalus ovis	33	12	2	15	23											10 Not found in buffalo	10	..	..	1
Haemonchus contortus	33	6	1	11	6											10 Not found in buffalo	10	..	..	2
Oesophagostomum columbianum	33	12	2	35	34	1	100	..	..	15						10 4	10 25	.. ..	.. ..	8 5
Oe. radiatum	33	27	2	40	11						3	33	..	..	16					
Gongylonema pulchrum	33	24	3	25	15	1	100	..	..	3						10 4	30 25	4 ..	18 ..	9 5
G. verrucosum	33	12	9	15	19											10 4	10 25	.. ..	.. ..	5 4–
Setaria digitata	33	15	1	9	5	1	100	..	..	4						10 Not found in buffalo	10	..	..	3
S. labiato-papillosa	33	24	1	3	2											10 4	20 25	1 ..	2 ..	2 3
Haemonchus similis	33	3	..	..	3															
Bunostomum bovis	33	6	1	2	2	2	50	..	..	1										
Mecistocirrus digitatus	33	12	3	6	7	1	100	..	..	1						10 4	50 25	5 ..	40 ..	24 4–
Oesophagostomum venulosum	33	3	1	1	1											10 Not found in buffalo	30	3	40	4

*Upper line of figures refers to cattle; lower line to buffaloes.

Seasonal variation

Very detailed data under this head is available only for Nagpur and it is summarized in Tables VI, VII and VIII.

TABLE VI

Seasonal variation in the incidence of infection—Host: Goat

Host: Goat	Cold season					Dry season					Wet season				
	October, November, December (1940, 41, 42), January (1941, 42, 43)					March (1941, 42, 43), April, May, June (1941, 42, 43)					July, August (1941, 42) September (1940, 41, 42)				
			Degree of infection					Degree of infection					Degree of infection		
Parasite	No. of animals examined.	Percentage of Infected animals	Minimum	Maximum	Average	No. of animals examined	Percentage of Infected animals	Minimum	Maximum	Average	No. of animals examined	Percentage of Infected animals	Minimum	Maximum	Average
Trematodes															
Cotylophoron cotylophorum	30	76	30	2025	120	41	38·4	3	500	71	30	13·2	13	90	
Gastrothylax crumenifer	30	26·4	2	40	22	41	7·2	9	60	24	30	16·5	4	320	128
Schistosoma spindalis	30	3·3	..	..	4	41	12	1	50	22	30	6·6	2	3	3
S. indicum	30	16·5	1	2	2	41	2·4	..	..	3	30	6·6	2	12	7
Fasciola hepatica	30	6·6	2	3	5	41	7·2	2	5	3	30	6·6	25	33	29
Paramphistomum cervi	30	3·3	..	..	5	41	4·8	60	80	70	30	6·6	30	40	35
Cotylophoron ovatum	30	13·2	2	7	4	41	7·2	4	13	9	30	6·6	67	67	67
Schistosoma bovis	30	3·3	..	..	2	41	4·8	2	33	18	30	3·3	..	..	6
Fasciola gigantica	30	13·2	1	4	2	41	14·4	2	40	9	30	19·8	1	10	4
Schistosoma hæmatobium	30		No infection			41	2·4	2	2	2	30	3·3	..	..	2
Cestoda															
Cysticercus ovis	30	56	1	20	5	41	43·2	1	6	4	30	6·6	3	3	3
Moniezia expansa	30	6·6	5	6	5	41		No infection			30	9·9	2	9	6
Moniezia benedini	30	6·6	1	4	2	41		No infection			30	3·3	..	..	4
Avitellina lahorea	30	10	2	11	8	41	24	4	50	29	30	3·3	..	..	12
Stilesia hepatica	30	16·5	3	86	31	41	11·2	3	70	22	30	16·5	4	50	22
Avitellina sudanea	30	13·2	1	3	2	41	12	1	50	32	30	3·3	..	..	4
Avitellina centuipunctata	30	10	3	5	5	41	11	1	68	26	30	13·2	3	8	5
Stilesia globipunctata	30	16·5	3	450	93	41	9·6	20	30	25	30	19·8	4	150	31
Stilessie vittata	30	26·4	2	112	4	41	38·4	4	70	41	30	13·2	10	16	13
Avitellina woodlandi	30		No infection			41		No infection			30	6·6	4	11	7
Cysticercus tenuicollis	30	6·6	1	3	2	41		No infection			30		No infection		
Echinococcus granulosus	30	3·3	..	..	1	41	4·8	2	3	2	30	3·3	..	..	17
Avitellina goughi	30	6·6	2	5	4	41		No infection			30		No infection		
Nematodes															
Gongylonema pulchrum	30	6·6	5	17	11	41	2·4	..	..	6	30	6·6	1	18	9
Bunostomum trigonocephalum	30	39·6	10	80	22	41	43	1	60	13	30	49·5	3	105	26
Gaigeria pachyscelis	30	30·3	4	80	19	41	14·4	6	25	15	30	19·8	4	27	10
Oesophagostomum radiatum	30	19·8	2	73	2	41	12	6	30	16	30	23·1	21	100	32
Haemonchus contortus	30	69·3	2	600	75	41	33·6	2	90	29	30	72·6	4	120	31
Oesophagostomum columbianum	30	13·2	10	85	10	41	43	5	200	52	30	43	4	56	24
Gongylonema verrucosum	30	13·2	2	7	4	41	11·2	6	15	9	30		No infeciton		
Trichostrongylus colubriformis	30	3·3	..	..	25	41		No infection			30	10	8	260	96
Cooperia curticei	30	6·6	3	30	16	41	16·8	1	40	22	30	6·6	4	6	5
Bunostomum phlebotum	30	10	7	68	9	41	2·6	5	60	8	30	19·8	6	12	18
Ostertagia ostertagi	30	3·3	..	..	5	41	7·2	10	150	22	30	6·6	15	69	42
Oesophagostomum venulosum	30	13·2	3	4	4	41		No infection			30	3·3	..	..	120
Strongyloides papillospum	30	3·3	..	..	76	41		No infection			30	3·3	..	..	120
Ostertagira circumciactata	30		No infection			41		No infection			30	3·3	..	..	8
Trichocephalus ovis	30		No infection			41	36	2	30	15	30	5·6	1	40	6

TABLE VII

Seasonal variation in the incidence of infection—Host : Sheep

Host : Sheep	Cold season					Dry season					Wet season				
	October, November, December (1940, 41, 42), January, February (1941, 42, 43)					March (1941, 42, 43), April, May, June (1941, 42, 43)					....				
			Degree of infection					Degree of infection					Degree of infection		
Parasite	No. of animals examined	Percentage of Infected animals	Minimum	Maximum	Average	No. of animals examined	Percentage of Infected animals	Minimum	Maximum	Average	No. of animals examined	Percentage of Infected animals	Minimum	Maximum	Average
Trematodes															
Cotylophoron cotylophorum	38	46·8	4	984	35	32	43·4	3	400	109	37	51·3	3	300	48
Fasciola hepatica	38	26	1	7	5	32	9·3	1	42	4	37	13·5	1	5	2
F. gigantica	38	2·6	..	..	4	32		No infection			37	5·4	4	19	11
Schistosoma indicum	38	15·6	4	15	7	32	28	1	24	5	37	10·8	1	12	6
S. spindalis	38	5·2	3	45	24	32		No infection			37	10·8	3	16	6
Gastrothylax crumenifer	38	26	3	320	87	32	15·5	1	200	46	37	13·5	2	696	74
Schistosoma bovis	38		No infection			32	9·3	2	5	2	37	5·4	5	7	6
Cotylophoron ovatum	38	2·6	..	..	3	32		No infection			37	2·7	..	..	5
Paramphistomum cervi	38	2·6	..	..	12	32		No infection			37	5·4	1	6	4
Schistosoma hæmatobium	38	2·6	..	..	4	32		No infection			37	2·7	..	..	1
Cestodes															
Moniezia benedini	38		No infection			32	6·2	4	20	12	37		No infection		
Cysticercus bovis	38	44·2	2	7	51	32	28	1	3	2	37	21·6	1	4	1
Stilesia globipunctata	38	15·6	1	15	5	32	6·2	15	35	25	37	24·3	1	400	71
Avitellina centripunctata	38	7·8	8	20	9	32		No infection			37	8·1	2	19	12
Stilesia vittata	38	20·8	1	96	4	32	21·7	2	2000	68	37	10·8	1	60	20
Avitellina woodlandi	38		No infection			32		No infection			37	5·4	8	50	29
Stilesia hepatica	38	18·2	5	21	13	32	12·4	3	60	22	37	29·7	1	105	63
Avitellina sudanea	38	2·6	..	..	4	32	3·1	..	..	3	37	8·1	3	8	5
A. goughi	38	2·6	..	..	13	32	3·1	..	..	15	37		No infection		
A. lahorea	38	7·8	8	14	11	32	6·2	3	7	5	37	8·1	7	210	10
Moniezia expansa	38	2·6	..	..	6	32		No infection			37	4·5	1	5	3
Cysticercus tenuicollis	38	5·4	1	3	2	32	3·1	..	..	5	37		No infection		
Echinococcus granulosus	38	7·8	3	3	3	32	6·2	2	4	3	37	5·4	1	4	2
Nematodes															
Oesophagostomum radiatum	38	39	1	52	3	32	24·8	2	50	56	37	40·5	3	258	53
Trichocephalus ovis	38	75·4	1	33	9	32	65·1	1	45	8	37	64·8	1	37	17
Bunostomum trigonocephalus	38	46·8	1	78	31	32	18·6	2	30	16	37	21·6	3	150	9
Hæmonchus contortus	38	85·8	2	300	48	32	34·1	2	50	24	37	81	5	500	83
Gaigeria pachyscelis	38	20·8	3	14	4	32	6·2	4	15	9	37	16·2	6	13	3
Oe. columbianum	38	46·8	1	62	19	82	12·1	4	90	30	37	29·7	1	70	19
Ostertagia ostertagi	38		No infection			32		No infection			37	2·7	..	..	62
Oe. venulosum	38	10·4	19	47	26	32	6·2	12	25	18	37		No infection		
Bunostomum phlebotum	38	7·8	3	80	44	32	6·2	4	60	32	37	16·2	5	15	5
Cooperia curticei	38	5·4	3	12	67	32	3·1	..	..	25	37	5·4	15	24	20
Trichostrongylus colubriformis	38	5·2	42	270	156	32	3·1	..	..	15	37	5·4	61	156	108
Gongylonema pulchrum	38	5·4	2	5	4	32	3·1	..	..	4	37	2·7	..	..	6
G. verrucosum	38	5·4	3	8	7	32		No infection			37	2·7	..	..	9

TABLE VIII

Seasonal variation in the incidence of infection—Host: Cattle

Host: Cattle	Cold season October—February					Dry season March—June					Wet season July—September				
			Degree of Infection					Degree of Infection					Degree of Infection		
Parasite	No. of animals examined	Percentage of Infected animals	Minimum	Maximum	Average	No. of animals examined	Percentage of Infected animals	Minimum	Maximum	Average	No. of animals examined	Percentage of Infected animals	Minimum	Maximum	Average
Trematodes															
Cotylophoron cotylophorum	11	91	66	4000	1750	15	66	50	1000	304	7	57	300	500	260
Fasciola gigantica	11	9	..	..	2	15	7	..	..	3	7	14	..	..	9
F. hepatica	11	63	30	1500	715	15	13	1	9	5	7	14	..	..	2
Gastrothylax crumenifer	11		No infection			14	54	5	700	324	7	29	250	300	275
Paramphistomum cervi	11	9	..	..	1	15	33	4	600	311	7	29	20	400	210
Schistosoma bovis	11	9	..	..	90	15	14	200	350	325	7	14	..	..	49
S. haematobium	11	45	41	900	304	15	20	20	300	182	7		No infection		
S. indicum	11	9	..	..	250	15	54	8	60	9	7	57	10	90	53
S. spindalis	11		No infection			15	13	100	200	150	7	29	20	80	50
Cestodes															
Moniezia benedini	11	9	..	..	6	15		No infection			7		No infection		
Moniezia expansa	11	6	2	6	4	15		No infection			7		No infection		
Cysticercus bovis	11		No infection			15	7	..	..	2	7		No infection		
C. tenuicollis	11		No infection			15	7	..	..	20	7		No infection		
Echinococcus granulosus	11	9	..	..	8	15	13	6	9	7	7	14	..	..	14
Nematodes															
Bunostomum bovis	11	9	..	..	9	15	6	..	..	1	7		No infection		
Gongylonema pulchrum	11	27	3	25	12	15	14	10	21	15	7	43	4	60	24
Gongylonema verrucosum	11	9	..	..	9	15	14	12	15	13	7	14	..	..	40
Hæmonchus contortus	11	18	1	11	6	15		No infection			7		No infection		
Hæmonchus similis	11		No infection			15		No infection			7	14	..	..	3
Mecistocirrus digitatus	11	18	1	9	5	15	7	3	6	4	7	14	..	..	4
Oesophagostomum columbianum	11		No infection			15	14	2	35	18	7	29	..	..	50
Oe. radiatum	11	54	1	38	9	15	7	..	..	5	7	29	8	40	24
Oe. venulosum	11		No infection			15		No infection			7	14	..	..	200
Ostertagia ostertagi	11	9	..	..	7	15		No infection			7		No infection		
Setaria digitata	11	9	..	..	1	15	20	1	9	6	7	14	..	..	5
S. labiato-papillosa	11	27	1	2	2	15	27	1	3	2	7	14	..	..	4
Trichocephalus ovis	11	18	15	48	31	15	7	..	..	5	7	14	..	..	4

Some important conclusions

Goats. In the Central Provinces and Berar, goats are found to be very heavily infected with Trematodes in Nagpur and the percentage of infected animals is greatest in winter season from December to February. Amphistome infection is the highest in all seasons and in winter as many as 76 per cent of animals are infected with them. *Cotylophoron cotylophorum* and *Gastrothylax crumenifer* are the commonest amphistomes. *Fasciola hepatica* and species of *Schistosoma* are not very common and the incidence of infection is also low. In Central India, the only trematodes found are species of *Schistosoma* and that too only in 16 per cent animals. Amphistomes are conspicuous by their absence.

In the Central Provinces and Berar, species of *Aviteleina* and *Stilesia* occur very commonly. In Central India *Stilesia vittata* is very common but the percentage of infected animals is only 24.

Hæmonchus contortus is found in the Central Provinces and Berar in about 73 per cent of animals in the rainy season but the percentage falls to 33 in summer and the average infection also falls down from 75 to 31 per animal. *Trichocephalus ovis* also occurs in a large number of animals but the infection is not heavy. In Central India, *Trichocephalus ovis* is more abundant occurring in as many as 81 per cent animals. *Bunostomum trigonocephalum* is also more common than in the Central Provinces and Berar.

Sheep. In the Central Provinces and Berar, sheep are more heavily infected at Raipur than at other places. *Cotylophoron cotylophorum* is the most common parasite occurring in Central Provinces in 51 per cent of sheep with an average load of 984 per infected animal. Species of *Schistosoma* and *Fasciola hepatica* are more abundant in sheep than in goats. But it is interesting to note that *Cotylopharon cotylophorum* is more abundant in the rainy season than in winter. In Central India, the only two trematodes infecting sheep are *Cotylophoron ovatum* and species of *Schistosoma*; but the infection is low; only 16 per cent of animals are infected.

Commonly the same cestodes which infect goat also infect the sheep. In the Central Provinces and Berar, *Stilesia globipunctata* occurs in about 17 per cent of the animals showing heavy infection. In Central India, the cestode infection is slightly more than the trematode infection.

Trichocephalus ovis and *Hæmonchus contortus* are the commonest Nematodes in sheep in Central Provinces. *Bunostomum trigonocephalum* and *Gaigeria pachyscelis* occur in 32 per cent and 16 per cent animals respectively but the degree of infection is low. *Gongylonema pulchrum* and *G. verrucosum* occur less frequently in sheep than in goats. In Central India, nematode infection is low and less varied. The only common nematodes are *Hæmonchus Contortus* and *Trichocephalus ovis*.

Cattle. In Central Provinces and Berar, Raipur is the most heavily infected area; as many as 75 per cent of cattle are infected with *Cotylophoron cotylophorum*, and at Nagpur 72 per cent are infected with this parasite. *Gastrothylax crumenifer* is found in 88 per cent cattle at Raipur and in 54 per cent cattle at Nagpur. The infection by *Paramphistomum cervi* is also heavy in Raipur but not so heavy as in Nagpur. The cattle in Central India are less heavily infected. Only 30 per cent of them harboured *Paramphistomum Cervi*, *cotylophoron cotylophorum* and *Fasciola hepatica*.

The cestode infection in cattle in the Central Provinces and Berar is confined only to *Moniezia expansa* and *M. benedini* and cysts of *Echinococcus granulosus*. The cattle at Mhow are also infected by these cysts.

The nematode infection in cattle in the Central Provinces and Berar is fairly varied but the incidence is low except for *Bunostomum trigonocephalum* which occurs in 43 per cent of animals. In Central India *Mecistocirrus digitatus* is found in 50 per cent cattle and *Gongylonema pulchrum* and *Œsophagostomum venulosum* in about 30 per cent.

Future work

As a result of this survey, the author thinks that work on the life-history and on preventive measures of the following parasites is very necessary at places noted against them—

- *Cotylophoron cotylophorum*—The Central Provinces and Berar: cattle, sheep and goats.
- *Gastrothylax crumenifer*—The Central Provinces and Berar: cattle, sheep and goats.
- *Schistosoma*—The Central Provinces and Berar: cattle.
- *Mecistocirrus digitatus*—Mhow: cattle
- *Stilesia globipunctata, Avitellina centripunctata*—The Central Provinces and Berar: goat, sheep.
- *Oesophagostomum venulosum*—Mhow: cattle.
- *Oe-radiatum*—The Central Provinces: goat, sheep.
- *Bunostomum trigonocephalum, Gaigeria pachyscelis*—The Central Provinces: goat, sheep.

Acknowledgements

In conclusion, the author wishes to record his gratitude to Mr H. B. Shahi, Director of Veterinary Services, Central Provinces and Berar, for valuable cooperation and to his research assistant, Dr B. S. Chauhan, for valuable assistance. Without these, this survey would not have been possible.

COMPOSITION OF *VANASPATI* (HYDROGENATED EDIBLE FATS) AND MARGARINE MANUFACTURED IN INDIA

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(Received for publication on 29 February 1944)

THE establishment of hydrogenated oil factories throughout India during the past decade and the rapid development of this industry that has taken place in recent years has greatly contributed to the increase in the production of edible fats and also their preservation and consumption in a better state. Even though large quantities of *vanaspati* are manufactured in India from several vegetable oils and are being sold in the Indian market under various brands, no data regarding the composition of *vanaspati* as a commercial product seem to have appeared in the literature which would give an idea of the usual ranges of analytical values in the different samples. The present investigation was undertaken and analyses of the various brands of *vanaspati* available in India have been made with the above object in view.

EXPERIMENTAL

Vanaspati samples were obtained from the various manufacturers in different parts of India through the courtesy of The Vanaspati Manufacturers' Association of India.

Most of the values were determined by the standard methods of fat analysis as described by the A. O. A. C. Iodine value was determined by the pyridine sulphate method and butyro-refractometer reading was taken at 40°C. Vitamin A was estimated in the unsaponifiable matter by the Carr-Price reaction. In all 26 *vanaspati* samples have been analysed and except for the Lion Brand (G), prepared by the Indian Vegetable Products, Limited, as a special product from coconut oil, they are all available in the market for the general public. In addition to *vanaspati*, Blue Brand margarine, manufactured as a substitute for butter has also been included in the investigation. The results of these analyses are presented in Table I.

TABLE I

Analysis of vanaspati

Sample No.	Details of the sample	Butyro-refractometer reading at 40°C.	Saponification value	Iodine value	R.M. value	Polenske value	Krischner value	Acid value as percentage of oleic acid	Peroxide value (as ml. of N/500 sodium thiosulphate)	Percentage of moisture
1	Dalda *vanaspati*	51·4	191·6	63·5	*nil*	0·35	*nil*	0·07	2·27	0·04
2	Bharat *vanaspati*, A	51·2	191·1	60·9	0·15	0·14	*nil*	0·13	..	0·02
3	Cotex *vanaspati*	51,0	194,8	62·2	0·21	0·31	*nil*	0·08	1·90	0·03
4	Bharat *vanaspati*, B	50·1	191·1	53·9	0·15	0·09	*nil*	0·05	..	Trace
5	Snow white, A	50,5	190·8	61·8	*nil*	0·09	*nil*	0·06	0·04	0·03
6	Snow white, A1	50·6	190·2	64,2	0·19	0·05	*nil*	0·08	..	Trace
7	„ B	49·8	190·6	64·5	0·10	0·09	*nil*	0·07	0·42	..
8	„ B1	50·3	190·2	60·7	0·10	0·04	*nil*	0·08	..	..
9	„ C	49·4	190·4	61·7	*nil*	0·09	*nil*	0·06	0·74	..
10	„ C1	51·6	191·0	58·9	0·21	0·10	*nil*	0·08	..	..
11	Lion Brand Grade A	50·3	190·6	61·1	0·20	0·05	*nil*	0·06	..	0·09
12	Modi *vanaspati* " Boat "	50·3	191·3	59·4	0·11	0·20	*nil*	0·14	0·56	..
13	Ganesh Flour Mills *vanaspati*	50·3	192·2	62·2	0·19	0·29	*nil*	0·08	[illegible]	..
14	Lion Brand Grade B	51·1	191·3	62·6	*nil*	*nil*	*nil*	0·05	..	0·02
15	Chamundi *vanaspati*, *Mysore*	51·1	190·2	68·9	0·22	0·20	*nil*	0·15	0·34	Trace
16	Lion Brand Grade C	50·0	191·3	58·0	0·40	0·19	*nil*	0·05	..	0·02
17	„ „ „ D	48·8	191·5	47·9	*nil*	0·09	*nil*	0·07	0·31	Trace
18	Tomco vegetable products, A	52·3	192·1	66·6	0·57	0·19	*nil*	0·21	..	..
19	Lion Brand Grade E	49·8	193·5	57·9	0·21	0·09	*nil*	0·10	0·68	0·02
20	Tomco vegetable products, B	48·1	188·5	62·7	0·52	0·15	*nil*	0·29	0·72	Trace
21	Lion Brand Grade F	49·6	190·7	55·9	0·21	0·09	*nil*	0·06	..	0·09
22	Tomco vegetable products, C	48·1	191·5	45·6	0·50	0·14	*nil*	0·05	0·46	0·01
23	Hindustan *Vanaspati* without aroma.	51·7	190·1	65·5	0·73	0·09	*nil*	0·09	0·78	0·06
24	„ „ with aroma	52·6	190·5	51·9	0·75	0·14	*nil*	0·11	0·90	0·03
25	Swastic *vanaspati*	50·0	203·7	59·0	0·85	0·25	*nil*	0·07	0·67	Trace
26	Lion Brand *vanaspati* G	34·6	257·4	1·0	8·05	15·21	2·25	9·06	..	Trace
27	Blue Brand margarine	39·9	239·5	22·6	6·60	9·77	1·1	0·14	3·63	14·1

It will be seen from Table I that the butyro-refractometer reading in all cases ranges from 48·1 to 52·6 with the exception of Lion Brand *vanaspati* (G) which gives a very low figure of 34·6, and Blue Brand margarine, which gives a reading of 39·9. Except for the last two samples, the range obtained is much above the figures for genuine butterfat (40·0-43·5). The saponification value ranges from 188·5 to 194·8 with the exception of three samples, viz. Swastik Brand *vanaspati* Lion Brand *vanaspati* (G) and Blue Brand margarine, which give rather high figures of 203·7, 257·4 and 45·6 respectively. The last two figures are very much above the values obtainable for pure *ghee.* Iodine value ranges from 45·6 to 68·8 with the exception of Lion Brand *vanaspati* (G) and Blue Brand margarine which give values of 1·0 and 22·6 respectively. It is rather striking that the Lion Brand *vanaspati* (G) gives such an exceedingly low value which is no doubt due to a higher degree of hydrogenation and also to the fact that it is made from coconut oil of low iodine value. For almost all the samples, the R. M. value varies from 0·0 to 0·85. But Lion Brand *vanaspati* (G) gives a divergent figure of 8·05 and Blue Brand margarine 6·60. Polenske value ranges from 0·0 to 0·85 in all the cases except Lion Brand, vanaspati Blue Brand margarine (G) which give very high figure of 15·24 and 9·77 respectively. Krishchner value is zero for all samples with the exception of the last two. It was also observed that none of the samples examined gave even a trace of colourto the unsaponifiable matter with the Carr-Price reagent thus indicating absence of Vitamin A. Nor could any traces of nickel be detected by the dimethylglyoxime test.

In chemical analysis fats are generally characterized by the determination of certain analytical constants by standard methods. However, since oils and fats are natural products, these values are subject to variations. The existence of a wide range of values in the case of certain constants in fats admits of their easy adulteration with other fats without detection. The Avelallemant process is considered to be a valuable auxiliary to the Reinchert-Meissl value in deciding whether butter fat is adulterated or not, and is based on the relative solubilities in water of the barium salts of the fatty acids.

The total barium value (A) of a fat is the equivalent of the saponification number expressed as barium oxide. The insoluble barium value (B) is that portion of the total barium value which represents the equivalent as barium oxide of the insoluble barium salts from 1 gm. of fat. In other words, it is the amount of soluble barium salt (as barium oxide) to be added to the potash soap from 1 gm. of fat necessary to precipitate completely the insoluble barium soap. The soluble barium value is (A-B), which is equal to say C, from which is calculated the value of B—(200+C). Butterfat is said to have practically a negative value by this formula whereas edible vegetable fats have a high positive value. This barium process is advocated in some parts of India to detect the adulteration of *ghee* with *vanaspati* or other animal fats. But before this process is generally adopted, it is very necessary to know the usual ranges of the barium value for the different brands of *vanaspati* as well as for *ghee* available in various parts of India. In the present investigation the barium values for the various brands of *vanaspati* manufactured in India have been determined and are presented in Table II. The method recommended in *Food Analysis* by Woodman was adopted for the determination of the barium value of fat.

The figures obtained from the formula B—(200+C) for all the samples of vegetable fats analysed are highly positive and range from 33·0 to 63·6. Our samples of *ghee* analysed gave negative values ranging from —2·8 to—11·0.

Summary

Twenty-six brands of *vanaspati* manufacture in India have been analysed for their physical and chemical constants. When compared to *ghee*, a lower saponification value (191·5), very low R. M. and Polenske values and a high butyro-refractometer reading (50·4) and Iodine value (59·2), generally characterize the *Vanaspati* products. The Krishchner value is nil for all the samples. There is, however, one exception to the above, viz. Lion Brand *vanaspati* (G), which along with Blue Brand margarine gave divergent results, namely, high Polenske and saponification values and a low butyro-refractometer reading.

The barium value of *vanaspati* ranges from +33·0 to +63·6, whereas in the case of farm produced ghee it ranges from —2·8 to —11·0.

Acknowledgement

Thanks are due to The Vanaspati Manufacturer's Association of India for arranging to supply the *vanaspati* samples and also to the various manufacturers for kindly furnishing the necessary details required regarding their samples.

N.B.—When this article was ready for publication, Ram, Gupta and Athawale (1943)*J. Indian chem. Soc.*, [Ind. and News Editions 6, 23] published the analysis of eight brands of *vanaspati* manufactured in India. Out of these only Lion Brand *vanaspati* was analysed by us. It will be noticed that Nehru Brand *vanaspati* with a saponification value 226·3, R. M., 6·38 and Polenske value, 6·17 analysed by them closely resembles samples 26 and 27 in Table I above.

TABLE II

Barium values of vanaspati

Sample No.	Details of the Sample	Saponification value	Barium value			B—(200+C)
			Total barium value A	Insoluble barium value B	Soluble barium value C	
1	Dalda	191·6	261·8	252·6	9·2	+43·4
2	Tomco Vegetable Product, A	192·1	262·5	250·4	12·1	+38·3
3	„ „ „ B	188·5	257·7	247·4	10·3	+37·1
4	„ „ „ C	191·5	261·8	250·3	11·5	+38·8
5	Hindustan Manufacturing Co., with aroma	190·5	260·4	253·5	6·9	+46·5
6	„ „ without aroma	190·4	260·1	254·1	6·3	+47·8
7	Bharat Vanaspati Co., A	191·1	261·2	249·3	11·9	+37·4
8	„ „ B	191·1	261·2	252·7	8·5	+44·2
9	Chamudni *Vanaspati*	190·2	260·0	250·2	9·8	+40·4
10	Modi (Boat brand) *Vanaspati*	191·3	261·5	253·5	8·0	+45·5
11	Cotex, Ghazibad	191·8	266·3	255·5	10·8	+49·4
12	Snow White Food-product, flavoured, A	190·2	260·0	246·5	13·5	+33·0
13	„ „ „ „ B	190·1	259·9	248·1	11·8	+36·3
14	„ „ „ „ C	190·0	261·1	251·4	9·7	+41·7
15	„ „ „ unflavoured, A	190·8	260·8	251·2	9·6	+11·6
16	„ „ „ „ B	190·6	260·6	250·9	9·7	+11·2
17	„ „ „ „ C	190·4	260·1	252·1	8·3	+43·8
18	Ganesh Flour Mills, *Vanaspati*	192·2	262·7	257·0	5·7	+51·3
19	Lion Brand Grade, D	191·5	261·8	270·3	8·5	+61·8
20	„ „ „ C	191·3	261·5	252·6	8·9	+42·7
21	„ „ „ F	190·7	260·7	253·2	7·5	+45·7
22	„ „ „ A	190·6	260·6	256·3	4·3	+52·0
23	„ „ „ B	191·3	261·5	249·3	12·2	+37·1
24	„ „ „ G	257·1	351·9	301·2	50·7	+50·5
25	„ „ „ E	193·5	261·5	259·2	5·3	+53·9
26	Margarine "Blue Brand"	239·5	327·4	295·5	31·9	+63·6
27	Farm Produced *ghee*	232·9	318·1	257·8	60·6	—2·8
28	„ „ „	224·9	307·4	248·2	59·2	—11·0
29	„ „ „	225·8	308·7	249·1	59·6	—10·5
30	„ „ „	218·1	298·7	247·4	51·3	—3·9

AVIAN TUBERCULOSIS IN A GOOSE

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(Received for publication on 23 November 1944)

Tuberculosis in fowls is an insidious chronic disease caused by *Mycobacterium tuberculosis avium.* But this disease has been regarded as being of very rare occurrence in ducks and geese. Feldman [1938] states that the disease is uncommon in water fowls, and ducks even when living in intimate contact with tuberculous fowls.

Eber [1925] found one case of tuberculosis among 460 ducks but failed to find the disease among 412 geese and 15 swans which he examined at necropsy. Klimmer [1930] over a period of 22 years examined 181 ducks and 145 geese and found tuberculosis in one per cent of the former and 1·4 per cent in the latter. Lænkholm as quoted by Zeller [1932] observed only one case of tuberculosis among 135 geese and 131 ducks.

Picard [1927] from the Dutch East Indies reported this disease in one goose and two fowls. Tuberculosis in fowls has been recorded from various parts of India but the extent of the losses caused by the disease is not yet known [Iyer, 1943]. There is no published record available to show the incidence of tuberculosis in ducks in India.

The object of this brief note is to record a case of tuberculosis in a goose. This is the first case of its kind encountered in Hyderabad State. It occurred in a flock of 13 geese and 17 ducks which were kept in captivity at the Zoological Public Gardens, Hyderabad-Dn. About 30 to 40 fowls were also in close contact with them. Later on, all contracted Ranikhet disease but 14 survived. Report of the sudden death of a goose was received by the writer when he was engaged in attending an outbreak of Ranikhet disease in the same gardens.

Post-mortem examination

It was an old bird showing marked emaciation. Extensive generalized tuberculosis involving most of the organs in the body was observed. To illustrate the macroscopic appearances of the infected organs the following notes are given :—

Liver. It was markedly enlarged. A few hæmorrhages were observed on the surface of the organ. Miliary foci of caseation and one large area as big as a play marble were seen in the right lobe. This large area of caseation was encapsulated and on section revealed cheesy material inside. The lesions showed great tendency to coalesce into large diffuse areas.

Spleen. It was 3-4 times the normal size with multiple lesions similar to those of the liver.

Lungs. Both lungs were involved and showed marked tuberculous lesions. Areas of caseation, extending around the bronchii and involving the pleura were seen. These lesions were similar to those described by Feldman [1938] and Dobson [1941]. These workers draw attention to the greater tendency for tuberculous lesions to occur in the lungs and on the serous membranes of ducks.

Heart. It was broader at the apex. Mortar-like black and white areas of necrosis were seen in the aorta and in the auricles in which numerous acid-alcohol-fast bacilli were present. The pericardium was also involved.

Gizzard. A few nodules on the serous membrane of the gizzard, especially over the portion which was lying in close proximity to the liver, were encountered.

Intestines. Ulcerated areas with round or rolled borders were found in the small intestine but nodules could be detected in the wall of the large intestine. The mucous membrane of the cloaca and cæca was thickened and corrugated.

In addition to the organs mentioned above lesions were also observed in the bones, ovaries and the air sacs.

Microscopic examination

Smears prepared from the lesions in the various organs when stained by Ziehl Neelsen's method revealed a large number of acid-alcohol-fast organisms closely resembling in morphology and arrangement those found in the tuberculosis of the fowls.

Confirmation of the material. Material from the soft tissues and bones collected from the specimen was forwarded to the Research Officer, Tuberculosis and Johnes Disease, Imperial Veterinary Research Institute, Mukteswar, who confirmed the diagnosis of tuberculosis and reported that the causal organism was of the avian type.

Samples of faeces from the incontact geese were examined microscopically for the evidence of the intestinal form of tuberculosis with negative results.

Tuberculin test. Thirteen geese, 17 ducks and 14 fowls were subjected to the intradermal tuberculin test. The wattle of fowls is selected as the most suitable site for inoculation but in ducks, geese and other birds, this organ is either absent or extremely small. Certain workers have recorded the results of their efforts to find a suitable site for the tuberculin test, including the conjunctivalsac, the eyelids, skin of the neck, breast wing and web of feet.

Avian tuberculin 0·1 c.c. was injected into the web of the feet in the geese and ducks but none reacted positively to this test. This method of testing in ducks and geese according to Craig and Davies [1937] is of no practical value in the diagnosis of tuberculosis. Dobson and others [1941] conclude that the tuberculin test carried out intradermally in the skin of the neck and in the cloaca and in conjunctiva with synthetic avian tuberculin was of no value in the diagnosis of tuberculosis in ducks.

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SELECTED ARTICLE

A REVIEW OF THE RESPIRATORY DISEASES OF THE HORSE

By JOHN FRANCIS, B. Sc., M.R.C.V.S.

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READING the older veterinary authors e.g. Smith, [1897]; Law, [1902]. Friedberger and Fröhner, [1908], one is struck by the scope and accuracy of their clinical and pathological observations. A vast amount of research, particularly in Germany and France, has been carried out on the respiratory diseases of the horse, but most of it was done before the days of motor transport: that is, before the methods of research into the ætiology of bacterial and virus diseases had been perfected, and as this group of diseases is particularly difficult to study it is not surprising that the subject is still somewhat confused. However, after having paid some attention to the question of predisposing causes and secondary bacterial invasion, an attempt will be made to assess the evidence that there are four respiratory diseases of the horse each primarily due to a specific virus infection.

It is far from easy to show experimentally that adverse environmental conditions will cause the onset of pneumonia, but all clinical observers appear to agree that they are of great importance in precipitating attacks of equine respiratory diseases. In a very fine clinical article on 'Pneumonia and Pleurisy in the Horse,' the late Sir Frederick Smith [1897] quotes Hunting as saying that 'want of condition, plus fast work, causes exhaustion, and then we only want cold, changeable weather to produce diseases of the lungs.' Smith believed exhaustion to be a 'direct exciting' cause of pneumonia, especially in young, soft horses, whereas older horses seem to possess a comparative immunity against some of the 'causes or conditions' which so freely contribute to the production of the disease in the young. Smith realized that the febrile attacks from which nearly all young horses suffer when first brought into stables may be associated with the process of acquiring immunity to the 'pneumonia microbe.' Pneumonia chiefly affects stabled horses and the great importance of good hygienic conditions is shown by the fact that five years after the introduction of 'sanitary science' into the French Army the annual incidence of pneumonia had dropped from 110 to 46 per thousand horses, and four years later to little more than $3\frac{1}{2}$ per thousand. Long rail and sea journeys also predispose to pneumonia. Coleman (Principal of the Royal Veterinary College, 1794-1839) laid great emphasis on the importance of well-ventilated stables, but unfortunately he did not display equally good judgement on many other subjects.

In parentheses, it may be said that the clinical methods of examining the chest, and the 'respiratory sounds,' described by Smith will be of great interest

to all clinicians working with large domestic animals. He found the phonendoscope to be by far the best instrument as an aid to diagnosis, and the extreme care with which he conducted his examinations can be judged from the fact that they were carried out at night with sawdust as bedding to 'insure quietude.' The best types of modern stethoscopes have been described in the *Lancet* [1941].

Koch [cit. M' Fadyean, 1938] found that normal horses harboured diplo-streptococci (see later) in the nasal passages. Boyer [1919], in an examination of the nose and mouth of normal horses, found that 8 per cent were carriers of '*Bact. Viscosus*' and 12 per cent of '*Str. pyogenes*' and '*Pseud. pyocyanea*' respectively. Hignett [1940] isolated group 'C' streptococci from the nasal cavity of normal horses.

There can be little doubt that one or other of a number of bacterial species may be present in the nasopharynx and produce disease when host resistance is lowered. Sporadic cases of pneumonia, nasal catarrh or strangles probably arise in this way, but severe outbreaks usually occur only when large numbers of young horses, or horses which have not previously been exposed to infection, are brought to remount depots or sales yards. The infectious nature of these outbreaks is probably due to a virus and, although the uncomplicated disease usually produces immunity, all observers agree that in itself it is very mild and may pass unnoticed. The severity of the symptoms and the mortality probably depend, therefore, on the virulence of the secondary organisms and above all on the care which is taken of the horses as soon as the first rise of temperature occurs. This was fully realized by Fitzwygram [1894], who showed that the 'incipient attack' could be most readily detected by the use of a clinical thermometer. The Royal Army Veterinary Corps made use of this fact during the last war, and as a result the losses from respiratory diseases were greatly reduced [Edwards, 1935b].

It will be seen from the foregoing summary that outbreaks of disease caused by any one virus may differ greatly in their severity and also in the clinical symptoms presented, and this is probably why there is confusion regarding the clinical classification of equine respiratory diseases; a confusion which was not apparently diminished by the experience of the last war. At that time, however, knowledge of human respiratory diseases was in no better state, and in 1918-19 influenza killed ten to twenty million people [Geddes-Smith, 1941]. On the other hand, it is well known that human 'influenza' may be a very mild disease, and the only satisfactory method of classification is on an ætiological basis. Considerable progress has already been made in this direction [Francis, T., 1941]. Our knowledge of equine respiratory diseases will probably only become clarified when they can be classified on an ætiological basis, and it can be definitely ascertained that a given outbreak of disease is due to a certain 'virus'.

Equine influenza

(Pink eye, influenza catarrhalis, typhoid fever, pferdestaupe)

'Malignant epidemics' which occurred about 1815 and the stringent methods which were employed to control them have been described by Youatt [1831]. Three out of five horses which were attacked died, and the clinical symptoms indicate that the disease was a severe form of equine influenza. He also describes equine influenza as it existed in this country during the latter part of the nineteenth century. Law [1902] has described in great detail the epizootic which paralyzed horse transport in the U.S.A. in 1872-73. Williams [1924] discussed other early epidemics and also described a severe outbreak in India in which the mortality in most groups of horses varied from 1 to 10 per cent but was occasionally as high as 20 per cent. Equine influenza caused serious inconvenience and loss during the last war [Todd and Soutar 1938; Udall, 1938; Anon., 1941], and it is still fairly prevalent in India.

Equine influenza and contagious pneumonia were confused by the early workers. The confusion that existed in the Army veterinary services was described by M' Fadyean [1889]. The two diseases had, however, been clearly differentiated by Falke, 1862 [see Friedberger and Fröhner, 1908]. The position was again confused by the work of Ligniers [see Nocard and Leclainche, 1903], who claimed that both diseases were primarily due to pasteurella organisms. Bemelmans [1921] also distinguished clearly between the two diseases, but emphasized that equine contagious pneumonia closely resembled human influenza. This similarity has also been described by Edwards [1935a]. It is felt, however, that no change should be made in the nomenclature of the equine diseases.

The clinical manifestations of influenza vary considerably, and detailed descriptions have been given by Law [1902], Friedberger and Fröhner [1908]. A characteristic feature is the marked prostration, and muscular and cardiac weakness during the early febrile stage. The temperature curve is much more erratic than in equine pneumonia. There is usually a harsh cough, the mucous membrances are reddened and there may be some nasal discharge. The conjunctiva assumes a deep red to mahogany colour, the eyelids are usually swollen and œdematous. The disease may not progress beyond this stage and the mortality is then very low, but under adverse conditions swellings are often seen in the subcutaneous tissues of the limbs and secondary intestinal or pneumonic symptoms develop; Law [1902] states that in

long railway journeys the mortality may be 100 per cent. It would appear that the influenza virus can attack any of the body tissues; there is always intestinal inflamation, and Friedberger and Fröhner [1908] state that the principal changes are in the organs of digestion. Apart from the virulence of the virus the type of disease depends on environmental conditions, management and on the species of the bacterial invaders.

Research work on the ætiology of influenza has been reviewed by Edwards [1935a] and by Todd and Souter [1938]. It is a highly infectious virus disease and may be set up by the subcutaneous inoculation of infected blood [this fact has been confirmed by Dale and Dollahite, 1939] or by direct or indirect contact. Saliva may be infective for at least eight months after recovery and it has been reported that a stallion transmitted infection by coitus for a period of six years. One attack of the disease confers a long-lasting immunity, but animals remain susceptible to equine contagious pneumonia.

Little attention has been paid to the problem of secondary bacterial invasion in equine influenza. It is probable, however, that in the pneumonic form streptococci and perhaps also staphylococci invade the lungs, whilst the good results obtained by Marshal and Lee [cit. Udall, 1938] following the use of pasteurella biological products suggest that this organism may also be of pathological importance. Krag and Tiedge [1938] isolated *S. enteritidis*, *Bact. viscosum equi* and *C. diphtheria* from the organs of horses affected with 'malignant strangles,' and it is possible that these organisms are of importance in the intestinal form of equine influenza.

EQUINE CONTAGIOUS PNEUMONIA

(Influenza pectoralis, contagious pleuropneumonia, brustseuche.)

The long incubation period (up to thirty days), irregular spread, lack of subcutaneous swellings or marked depressions during the early febrile stage and the regular temperature curve are said to differentiate this disease from equine influenza. There is often a yellowish discharge from the nose which is pathognomonic; a dry, husky cough may be heard. Friedberger and Fröhner [1908] and Bemelmans [1921] both state that there is intestinal inflamation, but manifest symptoms of intestinal disease are rare. Following the fall of the primary fever and particularly if the animal is worked, varying degrees of pneumonia and pleurisy may develop. The mortality may be as high as 20 per cent., and for many years the disease has caused serious losses amongst Army horses; the period of the last war was no exception [Udall, 1938].

The research work which established the ætiology of this disease has been reviewed by Edwards [1935a] and again by M'Fadyean [1938]. 'The disease has two causes which act separately and are responsible for different stages in the illness and different lesions...The virus is the veritable cause in the sense that it is responsible for the spread of the disease...' Law [1902] noted that neither Schütz nor Lignières had shown the experimental diseases produced by them, following the inoculation of bacteria, to be contagious and therefore did not accept their claim to have discovered the true cause of pneumonia or influenza. Gaffky and Lührs [1910-12, cit. Edwards, 1935a] showed that this disease was caused by a virus which could only be found in the lungs and at an early stage of the disease. After the primary rise of temperature the virus disappears from the lungs and the horse may recover without any very obvious signs of the disease. On the other hand, particularly if the horse is worked, bacteria may invade the lungs and a secondary rise in temperature with typical pneumonia will occur. It will be seen that this work anticipated the researches on human influenza by many years, and this was probably the first respiratory disease in which a 'dual causation' was discovered. Infection can only be transmitted by direct contact or by rubbing lung tissue from a horse in the early stages of the disease on to the nasal or buccal mucosa, and even then the incubation period may be as long as 39 days. This may explain why the disease has been considered to be non-infectious by some clinical observers, including Smith [1897], although the course of the disease as described by him suggests that he was dealing with brustseuche. Fraser [1938] has described what was probably an outbreak of this disease and states that streptococcal vaccines were valuable in reducing mortality. The experiments of Lührs [1928, cit. Edwards, 1935a] showed that animals which had received virulent lung tissue subcutaneously were resistant to infection by intranasal or intrabuccal rubbing of infective bronchial mucus which almost invariably produced disease in control animals.

The question of secondary bacterial invasion is somewhat confused. M'Fadyean [1938] assumed that the organisms isolated by Schütz [1887] and used in his attempts to produce brustseuche were typical streptococci. This appears to be incorrect, for an examination of the original article shows that the organism was a gram-negative bacterium which often occurred in pair and was usually encapsulated. Friedberger and Fröhner [1908] also state that the 'diplo-bacteria' of Schütz are gram-negative. It can be concluded with reasonable certainty that these were not pasteurella organisms. Nocard and Leclainche [1903] say that although the organism was originally described as gram-negative, it is now known to be gram-positive. This cannot be accepted, however, because his article on strangles [Schütz, 1888] makes it quite clear that he was

already (1887) perfectly familiar with the streptococci. Ostertag [1908, cit. Mitscherlich, 1941] also isolated gram-negative 'diplo-streptococci' from the lungs of horses affected with brustseuche. It can be concluded from the work of Mitscherlich [1941], which will be discussed later, that there is a gram-negative diplo-streptococcus which is pathogenic for the horse and which in some ways resembles a true streptococcus. It grows best in a medium containing blood or serum. On blood agar plates dry pin-point colonies surrounded by haemolytic zones are produced, and a flocculent deposit, leaving a clear supernatant fluid, is formed in 1 per cent serum broth. Continued cultivation is difficult and cultures usually die out. It is not pathogenic for guinea-pigs, but usually kills mice in from one to five days, and necrotic foci were sometimes observed in the liver. It would appear that this organism has several points of resemblance to the Neisseria group of organisms, which includes the meningococcus and gonococcus, as described by Topley and Wilson [1936].

Despite the foregoing discussion, streptococci were the ultimate cause of the pneumonia in the series of cases reported by M'Fadyean [1938], and recent work in this country and abroad has clearly demonstrated the important part played by group 'C' streptococci in respiratory and other diseases of the horse. Lignière's conclusions that pasteurella organisms were the primary cause of equine contagious pneumonia has already been mentioned. Webb [1909] isolated pure cultures of pasteurella organisms from the lungs of horses and donkeys dying from pneumonia, but the clinical history of the outbreaks suggests that the virus of contagious equine pneumonia was the primary ætiological agent. The extensive researches of Bemelmans [1921] indicate that, whilst pasteurella organisms may be isolated from cases of infectious pneumonia, diplo-streptococci, staphylococci or pseudo-pyocyanea are found more frequently.

It appears that under varying conditions of time and place any of the above organisms may complicate the virus infection of contagious pneumonia. It is therefore not surprising that there was great confusion at a time when bacterial classification had not been standardized, and in the early accounts it is difficult to decide with any certainty which organism is actually being described.

Infectious catarrh

(Equine infectious bronchitis, epizootic laryngetracheites, epizootic cough, Hoppegarte cough, Brussels disease, skalma)

Waldmann and Köbe [1936] described a highly infectious virus disease characterized clinically by a dry, strong cough as its chief symptom, and pathologically by lesions of bronchitis and peribronchitis. In horses kept under good conditions the disease usually ran its course as a pure virus infection, but when the preliminary symptoms were overlooked, and animals were not specially cared for, serious complications, such as broncho-pneumonia and pleurisy, occurred, due to invasion of the lungs by streptococci, sometimes followed by death. Horses were infected by the intranasal inoculation of lung filtrates and the disease was also transmitted to pigs and cattle. Recovered animals showed at least a partial immunity. Oprescu [1938] described a similar disease which could be transmitted by the subcutaneous inoculation of blood.

It is probable that infectious catarrh is similar to the condition known to the older German writers as skalma, and apparently various types of the condition are now prevalent amongst German army horses. Mitscherlich [1941] studied an outbreak affecting 176 out of 240 horses. He states that this disease can be differentiated from:

(1) Strangles by the presence of purulent nasal catarrh and the infrequency of lymph-node swellings and abscess formation.

(2) Brustseuche: in this disease there is a longer incubation period, a more prolonged fever and usually a red-brown nasal discharge and dull areas in the lungs. At autopsy there is a serious effusion and a thick fibrinous deposit on the pleura which is not seen in respiratory catarrh.

(3) Influenza, in which there is a marked morning recession of the fever, swelling of the conjunctiva, œdema of the breast and intestinal catarrh.

Blood taken during the febrile stage produced the disease when inoculated intravenously into another horse, but both the early serous and the latter 'grey-white' nasal discharge failed to cause the disease when introduced into the nasal cavity of a horse. Staphylococci were isolated from the serous nasal discharge, but when once it has assumed the characteristic grey-white colour gram-negative diplo-streptococci were found in about 50 per cent of the examinations. This organism was also isolated from the lungs of four horses which died of broncho-pneumonia. *Bacillus abortus equi* was also isolated from one of the cases and staphylococci from each of the others. A pure culture of the gram-negative diplo-streptococcus produced purulent rhinitis in a horse which had just recovered from a simple virus infection. It was concluded that this organism, the chief characteristics of which have already been described, was the most important secondary bacterial invader and that it is probably similar to an organism isolated by the early workers from cases of brustseuche. Traub [1941] isolated group 'C' and Seeleman [1941] groups 'C' and 'D' streptococci from cases of this disease.

Beller and Traub [1941] have made a careful study of respiratory catarrh and in general their findings agreed with those of Waldmann and Köbe [1936]; they were, however, unable to transmit the disease to cattle, ferrets, white mice or pigs, or to propagate it in tissue culture.

Bazely and Battle [1940] have described a disease which rapidly reached epidemic proportion when numbers of horses were brought into close contact for more than ten days. Various forms of the disease were observed, but they were grouped under the general term 'respiratory catarrh'. The authors apparently believed the disease to be due to group 'C' hæmolytic streptococci, but horses that had passed through an epidemic were immune to subsequent exposure, and it is quite possible that the condition was similar to the disease described by Waldmann and Köbe and was primarily due to virus infection. Walker [1935] has described a highly infectious epidemic of coughing amongst horses in Great Britain.

Pulles [1941] and Rexroth [1941] state that the intravenous injection of 'Thoromangan,' an iodine-thorium-manganese compound, is valuable in the treatment of infectious catarrh.

Strangles

The incidence and severity of strangles vary in different parts of the world. It is a serious disease in the remount depots in India. Arnold [1931] has described the disease in that country and the methods of prophylaxis and treatment which are employed. Following a primary inflammation of the upper respiratory tract an inflammation of the submaxillary lymph-nodes develops which is not necessarily followed by abscess formation. Pus taken during the earliest stages of the disease contains no micro-organism.

Wooldridge [1936] mentions that a virus may be the primary cause of strangles, but most writers appear to accept the view that it is caused by *Str. equi*. Bazely and Battle [1940] say: 'We have found no suggestion of a virus in this disease, and, judging from the regularity by which *Str. equi* occurs, none appears to be needed for an adequate ætiology'. The fact that *Str. equi* but not the other types of group 'C' streptococci usually associated with equine disease, can nearly always be isolated from unruptured matured abscesses lends support to this view, but the conclusion that *Str. equi* is the sole cause of strangles cannot easily be accepted on epidemiological grounds.

It is well known that strangles is a disease of young horses and a life-long immunity is usually produced. It is notoriously difficult to produce immunity against streptococci and success has not been attained by the use of streptococcal vaccines to prevent strangles [Edwards, 1935]. The highly infectious nature of the disease and the fact that a strong immunity is usually established suggest that it is primarily caused by a virus. Bazely and Battle state that their work was 'conducted in a laboratory which for the past two and a half years had concerned itself exclusively with the study of hæmolytic streptococci,' and we can only conclude that no attempt was made to isolate a virus.

Clinical observation and experiments led Arnold [1933] to conclude that strangles is primarily due to a virus which produces febrile reactions only; a secondary invasion by streptococci produces all the recognized manifestations of the disease. The virus is responsible for the high infectivity and a benign uncomplicated virus infection is sometimes observed. On the other hand, sporadic cases may be due to bacteria only. The virus disease can be produced by the intra-tracheal inoculation of filtered strangles pus and this leaves the animal solidly immune to subsequent infection. A formalized filtrate produced only slight immunity.

Van Dorssen [1939] concluded that 'benign strangles' was caused solely by *Str. equi* Richters [1935] isolated true diphtheria bacilli from the unopened lymph-nodes and nasal discharge of some horses affected with malignant strangles; injections of these organisms into susceptible horses produced inflammation of the nasopharyngeal mucosa and severe gastro-enteritis. He concludes, however, that neither these organisms nor streptococci are the primary cause of strangles, but that it is due to a specific virus. It has not been possible to find any subsequent publication of Richters' substantiating this view, but Beller and Traub [1941] and Rexroth [1941] reached the same conclusion. Law [1902] noted that, although horses inoculated with strangles streptococci developed abscesses, there was no evidence that the disease produced was contagious, but taken as a whole the evidence suggesting that a virus is the primary ætiological agent in this disease is not perhaps so strong as in those already described.

It has already been mentioned that Krage and Tiedge [1938] isolated *S. enteritides, Bact. viscosum equi* and *C. diptheria* from the organs of horses affected with malignant strangles.

Lesions in Nervous and Other Tissues

The conditions described in this review are usually accepted as being respiratory diseases, but one cannot help remarking that the primary virus infection often exhibits all the features of a generalized disease. There may be marked impairment of kidney and liver function, and the predilection of the influenza virus for the intestine is well known. Traub [1941] and Rexroth [1941] noted pustular and exanthematous lesions during the course of infectious catarrh, and in an exhaustive clinical study Steffan [1941]

demonstrated partial or complete auricular-ventricular heart block.

The nervous and psychic disturbances which may occur during the course of respiratory diseases have been noted by most authors. Bemelmans [1921] attributed many of the primary disease symptoms to nervous derangement, and Steffan [1941] has made a special study of the subject. There can be little doubt that there is a relationship between the neural damage caused and the subsequent onset of roaring, stringhalt and the other nervous affections of the horse. Ebberbeck's [1940] pathological investigations have provided much evidence in support of this view, but it is probable that nutrition [Mitchell, 1936] and heriditary disposition also play their part.

Discussion

The four respiratory diseases have been described as though they were separate entities, and some of their main features are summarized in Table I, but it must be admitted that, whilst the typical diseases can be recognized, there are many outbreaks which even experienced workers are unable to classify [Theiler, 1918].

Table I

—	Contagious pneumonia	Influenza	Infectious respiratory catarrh	Strangles
Incubation period .	Long, up to 40 days . .	Short	Short	Short.
Infectivity . .	Low, only by direct contact. Spread is irregular and the duration of an outbreak in a group of horses is much longer than with the other diseases.	High, by direct and indirect contact. May assume very severe epidemic proportions.	High, by direct and indirect contact.	High, by direct and indirect contact.
Symptoms . .	Primary fever with regular temperature curve may pass almost unnoticed. Pneumonia may then develop, accompanied by a secondary rise of temperature. A yellow discharge from the nose is said to be pathogomonic.	Primary fever with larger diurnal variations of temperature marked depression, cardiac and muscular weakness. Reddening of mucous membranes and swelling of subcutaneous tissues. Severe intestinal, or less often respiratory involvement may follow.	Fever, purulent rhinitis, coughing and occasionally swelling of the sub-maxillary lymphnodes. Pneumonia may occur under adverse conditions.	Fever, early rhinitis followed by marked swelling and often abscess formation of the sub-maxillary lymphnodes. Pneumonia may occur under adverse conditions.
Action of neosalvarsan	Effective	Not effective . . .	Not effective . . .	Presumably not effective
Mortality . .	Higher than in the other diseases 10-20 per cent.	Usually low . . .	Low	Usually low.

It would appear that infectious pneumonia can be differentiated from the other diseases by its longer incubation period and lower infectivity; the clinical observation that there is no cross-immunity between infectious pneumonia and influenza supports this view. Bemelmans [1932] wrote a long review comparing human influenza, brustseuche and canine distemper. Many of his views can no longer be accepted, but the review is valuable in that there are 345 references to the older literature. Lührs [1933] commented on Bemelmans' article. They both agreed that brustseuche and influenza were distinct diseases, but it is probable that Bemelmans [1933] described infectious catarrh as 'abortive brustseuche,' and other writers have stated that outbreaks of catarrh predispose to brustseuche. Lührs [1933], however, is quite definite that they are separate diseases; the whole course of an outbreak of infectious catarrh is much shorter and neosalvarsan exerts no therapeutic action.

Discussing strangles, Percival [1834] said: 'I now begin to doubt whether any tumour or abscess at all is absolutely necessary........I believe most horses to have sooner or later in life, *strangles fever;* but I doubt that everyone that has that fever has it demonstrated or accompanied by local tumour or abscess........Of the many young three to four year-old horses which have in the course of years passed under my medical superintendance, I should say that not have above one in four of them had undergone regular strangles, though very many of them at one time or the other had sickened........ Let other veterinarians turn their attention to these interesting points: we shall then probably, ere many years pass over our heads, elicit some curious if not valuable addition to our present stock of

knowledge on the subject'. Pillers and others (1934) also discussed the difficulty of differentiating strangles from the various catarrhal affections of horses.

Beller and Traub [1941] noted that strangles and infectious catarrh might occur together in the same horse and after a very careful study put forward the interesting suggestion that they are both primarily due to the same virus and that the different symptoms are due to different secondary invaders or predisposition of the horse. Rexroth [1941] as a result of clinical and pathological investigations, supported this view.

Lührs [1933], whilst admitting that no one can mistake true epidemic equine influenza, states that there are many outbreaks of disease which one cannot easily classify, and Law [1902], who was obviously familiar with the epidemic disease, says that in the slighter cases the lesions are often confined to the anterior part of the respiratory organs which are congested and red, the lesions may extend to the bronchi. In human medicine there are similar differences between epidemic and sporadic influenza, and, although in some outbreaks influenza 'A' or 'B' virus can be identified, in other clinically identical outbreaks no virus can be isolated.

It would appear that, whilst the main clinical entities—strangles, infectious catarrh and influenza —can be recognized, we still do not know whether they are caused by closely allied or distinct viruses.

SUMMARY AND CONCLUSIONS

An attempt has been made to assess the evidence concerning the ætiology of the equine respiratory diseases, and it is tentatively concluded that contagious pneumonia is a distinct entity primarily due to a virus. There is no doubt that viruses are also responsible for outbreaks of infectious catarrh and influenza, and probably also of strangles.

It appears possible that strangles and infectious catarrh are both primarily due to the same or a similar virus, and there is no evidence which proves beyond doubt that the virus of influenza is distinct.

In any of the above diseases the uncomplicated virus infection may pass almost unnoticed, although producing immunity to subsequent infection. Climatic conditions and methods of management, however, have a great influence on the course of the disease; under adverse circumstances, a variety of bacteria may produce serious complications, and there is little doubt that individual animals may develop more or less characteristic symptoms when suffering from a simple bacterial infection.

The results reported by Stableforth [1939], Hignett [1940], Hignett and King [1940], as well as other workers abroad, has demonstrated the value of the sulphonamide group of drugs in the treatment of pneumonia. The apparent specific action of neosalvarsan and the sulphonamide group cannot, however, be accepted as evidence that bacteria are the primary ætiological agents. Methods of immunization, if they could be evolved, might be effective and economical.

During the last great war it would appear that research was carried out by a few enthusiasts, but Edwards [1935b] has said that the need for a trained research cadre attached to the R.A.V.C. was apparently not realized until the end, and it never came into being; a great opportunity to investigate the respiratory disease of the horse was lost.

Knowledge concerning the viruses, hæmolytic streptococci, methods of drying and preserving biological materials, and the technique of investigating respiratory diseases has greatly improved since the last war. It is also felt that one of the three methods which have been used to prepare swine-fever vaccine [see Francis, J. 1941], two of which have also been successfully used in the control of rinderpest, might very well be adapted particularly for the preparation of an equine influenza vaccine. In this disease, and possibly also in infectious catarrh, virus is known to circulate in the blood. It appears, therefore, that if the need arose, and the necessary facilities were granted, an efficient research cadre working in conjunction with clinicians could do much to clarify our knowledge of equine respiratory diseases and possibly evolve methods of specific immunization. One gains the impression that the clinical and pathological investigations which are being carried out by the German Army veterinary services are of a high standard. It is pleasing to note [J.A.V.M.A. 98 : 254] that progress is being made, at the U.S. Army Veterinary Corps Laboratory, Front Royal, in the study of various respiratory diseases of the horse and that vaccines against influenza and strangles are being developed.

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ABSTRACTS

Some peculiarities of ruminant nutrition. Harold Goss (1943). *Nutrition Abstracts and Reviews 12*

The author in pointing out the peculiarities of ruminant nutrition has shown that micro-flora in the rumen can (*a*) make complex carbohydrates of feeds available by splitting them up into simpler products, (*b*) synthesize vitamin B complex and vitamin K, which are therefore not required to be supplied in the food. Vitamins like A and D which are not synthesized are, however, required to be supplied in the diet. The microflora can also convert non-protein nitrogenous substances, such as urea and ammonium salts, into bacterial protein which later undergoes normal digestion in the true stomach.

Although ruminants generally live on bulky foods, they can be maintained on an exclusive dietary of concentrates. [N.K.]

The occurrence of hæmolytic cocco-bacilli in the nose of normal sheep and cattle. T. J. Bosworth, and R. Lovell (1944). *J. Comp. Path.* 54, 168.

Gram-negative hæmolytic cocco-bacilli have been recovered from pneumonic conditions in sheep and cattle, both in Great Britain and America. The authors recovered similar organisms from the noses of 26 of 51 ewes examined by them in a flock of 199, half of which were affected with nasal catarrh following a spell of cold weather. As the significance of these findings was difficult to assess, the examination was extended to include a number of healthy sheep and cattle. Accordingly, they swabbed the noses of 100 normal healthy sheep, 50 from each of two flocks, and the lower portion of the trachea of 84 sheep shortly after slaughter. They also took swabs from the nose of a total of 113 cows and calves in three different herds. The swabs were rubbed on five per cent ox-blood agar plates, and after 24 to 48 hours incubation, suspicious colonies were picked and examined. The results are given in the table below, which also includes those from the examination of the 51 sheep affected with nasal catarrh.

Number and species of animal	State of health	Site of swab	Number of isolations
51 sheep	Nasal catarrh	Nose	26
50 sheep	Healthy	Nose	26
50 sheep	Healthy	Nose	14
84 sheep	Healthy at p.m.	Lower portion of trachea	7
6 cattle	Healthy	Nose	0
49 cattle	Healthy	Nose	6
50 cattle	Healthy	Nose	3

Omitting the sheep which were suffering from nasal catarrh hæmolytic cocco-bacilli were recovered from the nose of 40 of 100 healthy sheep and from the trachea of 7 of 84. The number of cattle from which hæmolytic cocco-bacilli were isolated was much smaller, only 9 out of 105 (113 ?). From this it appears that cocco-bacilli of this class are commonly present in the nasal passages of sheep and to a less extent in those of cattle. Their recovery from pneumonic and other respiratory affections in sheep and cattle should not, therefore, be taken to mean that they are responsible *per se* for such conditions. It is more probable that their role is only secondary to some primary factor which may be either a virus, as suggested by some, or change in environment.

The characteristics of these organisms were as follows :—Gram-negative cocco-bacilli or short rods, non-motile, producing a narrow zone of hæmolysis on ox-blood agar plates, usually fermenting glucose, maltose, mannite and saccharose and sometimes lactose, producing no change in litmus milk, and not forming indole except the cattle strains, about half of which did so. (Organisms with the general characters of the cocco-bacilli above described have often been grouped with the *Pasteurella*, but their exact taxonomic position is doubtful.) [L.S.]

Isolation of *Pasteurella septica* from an appendicular abscess. G. B. Ludlam (1944). *J. Path. Bact.* 56, 307

This article records the isolation of *Pasteurella septica* from the appendicular abscess of a sempstress working in a hospital in Edinburgh. The abscess which was about the size of a cherry and situated in the proximal part of the appendix contained yellowish creamy pus, microscopical examination of which showed numbers of pus cells and a few gram-negative bacilli, some of which appeared to be intracellular. Culture on blood agar yielded a profuse pure growth of a gram-negative cocco-bacillus which culturally and biochemically was found to resemble *Pasteurella septica*. The organism proved pathogenic for the guinea-pig, rabbit and mouse but only slightly so for the fowl. The identity of the organism was further confirmed by agglutination and agglutinin absorption tests with known strains of *Pasteurella septica*.

A specimen of the patient's serum obtained two months after the operation agglutinated a suspension of the organism to a titre of 1 : 240 and a known strain of *Pasteurella septica* to 1 : 60. An explanation is offered as to this discrepancy.

During the previous three months there had been nine other cases of appendicitis among nurses at this hospital but only three of these were examined bacteriologically and they yielded only *Bact. coli* and *Cl. welchii*. The sera of some of these patients also proved negative for the presence of agglutinins to *Pasteurella septica* and the isolated strain. It would thus appear that the case under review was an isolated one. No explanation could be offered as to how the patient had got infected.

The author claims that this is the first authentic record of *Pasteurella septica* infection in a human being in Great Britain ; for though *Pasteurella*-like organisms were isolated from a number of patients suffering from infections of the gastro-intestinal tract during the last Great War, they were not sufficiently studied to establish their true identity. Elsewhere, mostly on the continent of Europe, the organism has been recovered from the following conditions :—prolonged puerperal pyrexia, gastro-enteritis following the handling of fowls suffering from *Pasteurella* infection, empyæma, meningitis following the use of rabbit-muscle as a hæmostatic in an intracranial operation, meningitis following cranial fracture, blood infection following a panther bite, local abscess as a result of cat bite and the bite of a rabbit. [L.S.]

Infection of cat-bite and dog-bite wounds with *Pasteurella septica*. E. N. Allot, R. Cruickshank, Ruth Cyrias-Williams, V. Glass, I. H. Meyer Edith A. Straker and G. Tee (1944). *J. Path. Bact.* 56, 411

This is a record of six cases of dog and cat-bite wound infection (three dog-bites and three cat-bites) with *Pasteurella septica*. Infection of cat-bite wounds with *Pasteurella septica* has been recorded before (see the abstract printed above), but this is the first time a similar observation has been made in regard to dog-bites. This adds the dog to the probable list of *Pasteurella* carriers.

In three of the six cases, osteomyelitis occurred as a complication. This suggests that *Pasteurella septica* has a special affinity for bone which probably accounts for the intractable nature of most animal bites.

The article is of interest as throwing further light on the existence and importance of *Pasteurella* carriers. [L.S.]

A new treatment for dracontiasis. M. Elliott (1942). *Trans. roy. Soc. Trop. Med. Hyg.* 35, 291-301

After brief accounts of the history, geographical distribution, incidence, pathology, diagnosis and the clinical types of dracontiasis, the author deals with the details of treatment. Accordng to him, the ideal treatment for this disease would be to introduce a drug into the tissues which would kill the worms, thus facilitating their extraction or else leading to their rapid dissolution. Phenothiazine has all this action

ORIGINAL ARTICLES

A COMPARATIVE STUDY OF RINDERPEST BULL-GOAT-VIRUSES, WITH A BRIEF SURVEY OF THE WORK DONE WITH RINDERPEST GOAT VIRUS IN MADRAS

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With the inauguration of a Serum Institute at Madras, much work has been done to evolve a safe, efficient and cheap method of combating rinderpest in this province. Rinderpest virus, modified by serial passage through goats, was given an extensive trial both at the laboratory and in the field and such modified virus has now been employed for the last ten years.* Saunders and Ayyar [1936] were among the first to put the method of immunizing cattle by the goat virus alone method to a critical and controlled test with a view to determine whether successive passages of virus through goats would so alter the virus as to make it safe for use as a vaccine for protecting cattle against the disease without anti-serum. D'Costa [1938] says that goat virus causes a 70 per cent mortality in hill bulls and recommends that highly susceptible animals should be immunized by the sero-virus method, employing 'fixed' goat virus as the infective agent and slightly reducing the dose of protective serum; while Bachan Singh [1939] states that the 'fixed' virus from Mukteswar possesses a low virulence for cattle of indigenous breed and that it has been found to be safe for local-breed buffaloes of Central Provinces and Berar.

Workers in other provinces have reported favourably on the use of the goat virus alone method in combating rinderpest, but the experience gained in this province goes to show that this method could not be advocated for wide use, as it produces undesirable reactions and other bad effects after inoculation. The object of this paper is to place on record a brief survey of the work done in this province with goat virus and to make a comparative study of rinderpest bull virus and rinderpest goat virus, with a view to find out whether there is any attenuation of the virus when maintained in successive passages through goats.

Rinderpest goat virus

The goat virus is maintained at the Serum Institute by successive passages in local breed of goats. Two strains of virus, viz. (1) The Madras strain and (2) the Mukteswar strain, were maintained. After running the two strains for over seven years when more than 400 passages of each strain were completed, the two were amalgamated together and maintained as a single strain, Madras strain No. 2, as it was found that there was not much difference in the degree and percentage of reactions set up by them in the control buffalo-calves. In the meanwhile a quantity of 'fixed goat virus' was obtained from Mukteswar and that is also now being maintained as 'Mukteswar strain No. 2.'

The two strains are periodically tested for every tenth passage on young susceptible buffalo-calves of local breed. Most of the goats used for virus production, are invariably bled to death to meet the demand for virus. The control calves are observed for reactions and then tested with a dose of virulent bull virus, after the reactions to original inoculation of goat virus, have subsided.

So far about 375 passages of the Mukteswar strain No. 2, and 225 passages of Madras strain No. 2 have been completed. One hundred and thirty-nine calves for the Mukteswar strain and forty-two for the Madras strain have been used and the observations on the type of reactions amongst them are summarized below.

Mukteswar strain No. 2. Out of the 139 calves, 116 or 83·5 per cent had thermal reactions, 86 or 61·9 per cent had diarrhœa, and 39 or 28·1 per cent had mouth lesions also. There was a mortality of 32 calves or 23 per cent amongst them. Subsequent immunity tests on the surviving calves showed that they had acquired immunity.

Twenty-one calves showed no reaction to the original injection with goat virus and on subsequent test conducted on 19 calves with virulent bull virus, 11 proved to be non-reactors and immune throughout. Seven had a thermal reaction, four had diarrhœa and two of them mouth lesions also. Three of them died of reactions to re-test.

Madras strain No. 2. Out of 42 controls used for this strain, 40 or 95·2 per cent had thermal reactions, 31 or 73·8 per cent had diarrhœa and 16 or 38·1 per cent had mouth lesions also. There was a mortality of 7 or 16·7 per cent amongst them. Immunity tests on the calves that survived showed that they had acquired immunity. There were practically no non-reactors to the original injection with goat virus.

*Annual Reports. Civil Veterinary Department, Madras, 1932-33 to 1943-44.

TABLE I

Type of reaction observed amomg goat virus controls—Mukteswar Strain No. 2

Number of passages*	Number of control calves used	Type of reactions observed					Reactions after retest		Remarks
		Thermal	Diarrhœa	Mouth lesions	Mortality	Number of non-reactors	Among the reactors to original injection	Among the non-reactors	
(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)
1 to 25	55	50	34	11	11	6	Thermal in one	Thermal in one	
26 to 50	14	12	8	7	5	2	*nil*	*nil*	
51 to 75	4	4	2	1	1	..	*nil*	*nil*	
76 to 100	8	4	2	..	..	4	Thermal in one	Diarrhœa in one	
								Thermal and diarrhœa in one	Both died
								No reactions in others	
101 to 125	4	3	3	2	1	1	*nil*	*nil*	
126 to 150	6	5	5	4	2	1	*nil*	Thermal, soft faeces and mouth lesions in one	Died
151 to 175	6	4	1	..	..	2	*nil*	Thermal in two	
176 to 200	6	4	1	..	..	2	*nil*	Thermal, diarrhœa and mouth lesions in one, and thermal and diarrhœa in the other	
201 to 225	6	4	3	1	1	2	*nil*	Not retested	
226 to 250	6	6	6	2	1	..	Not retested	do	
251 to 275	2	2	2	2	2	..	do	do	
276 to 300	6	5	4	1	1	1	*nil*	*nil*	
301 to 325	6	4	6	3	4	..	*nil*	*nil*	
326 to 350	6	5	5	3	3	..	*nil*	*nil*	
351 to 375	4	4	4	2	..	..	*nil*	*nil*	
Total 375 passages	139	116	86	39	32	21			
		83·5 per cent	61·9 per cent	28·1 per cent	23 per cent				

*Groups of twenty-five passages each are taken serially and classified.

NATURE OF REACTIONS OBSERVED

In both the strains, most of the reactors developed a high temperature, varying from 103°F. to 105°F., from about the fourth day. The temperature persisted for some days; dullness, inappetence, suspension of rumination, nasal catarrh and catarrh of the eyes were also observed. In many cases of severe reactors diarrhœa set in from about the sixth or seventh day, which in most of the fatal cases was fetid, shooting and watery and persisted till

death. Those that recovered after diarrhœa were much emaciated. Congested buccal mucous membrane was observed in many cases from about the seventh day and, in some, ulcers were also noticed on the eighth or ninth day. In most of the cases congestion persisted for about a week. In moderate cases, only a thermal rise was observed for three or four days followed by passing of soft faeces for two or three days and the animals gradually returned to the normal condition.

On autopsy the striking lesions observed were the acute gastro-enteritis and occasional ulceration of abomasum and cæcum.

Animals which recovered from a severe type of reaction were observed to be weak and much emaciated.

Field trial

Experiments were first conducted during the year 1932-33 in ten selected villages with goat virus alone and, judging from the encouraging results obtained in the experimental camps, the method was given a wide trial for some years in clean areas. Some inoculations in affected areas were also performed. Table III shows the number of inoculations performed by this method and the mortality recorded amongst them. During the year 1935-36

TABLE II

Type of reaction observed among goat virus controls—Madras Strain No. 2

Number of passages*	Number of control calves used	Type of reactions observed					Reactions after retest	
		Thermal	Diarrhœa	Mouth lesions	Mortality	Number of non-reactors	Among the reactors to original injection	Among the non-reactors
(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)
1 to 25	4	4	1	1	..	..	*nil*	*nil*
26 to 50	4	4	3	1	..	..	*nil*	*nil*
51 to 75	4	3	3	3	1	..	*nil*	*nil*
76 to 100	4	4	4	..	2	..	Not retested	Not retested
101 to 125	6	6	4	..	..	..	do	do
126 to 150	6	5	4	2	..	..	*nil*	*nil*
151 to 175	4	4	3	2	2	..	*nil*	*nil*
176 to 200	6	6	5	5	2	..	*nil*	*nil*
201 to 225	4	4	4	2	..	..	*nil*	*nil*
Total 225 passages	42	40 95·2 per cent	31 73·8 per cent	16 38·1 per cent	7 16·7 per cent	..		

*Groups of 25 passages each are taken serially and classified.

TABLE III

Number of 'goat virus alone' inoculations performed and the mortality amongst them (Cattle and buffaloes only)

Years	Number of inoculations performed			Number of inoculated animals dying of the disease		
	In clean areas	In affected areas	Total inoculations	Among those inoculated in clean areas	Among those inoculated in affected areas	Total deaths
(1)	(2)	(3)	(4)	(5)	(6)	(7)
1932-33	129	..	129	1	..	1
1933-34	2,640	..	2,640	4	..	4
1934-35	32,911	..	32,911	22	..	22
1935-36	61,114	..	61,114	88	..	88
1936-37	62,821	84	62,905	51	3	54
1937-38	26,741	3,756	30,497	23	..	23
1938-39	10,934	..	10,934	14	..	14
1939-40	1,294	..	1,294	..	..	..
1940-41	301	..	301	..	..	..

0·1 per cent among the inoculated were reported to have died as a result of the severe reactions set up by this method and the mortality had occurred mostly among buffaloes, which class of animals was found to be less resistant to the effects of this method of inoculation. During 1936-37 out of 62,905 animals inoculated with goat virus, 54 animals, mostly buffaloes, were reported to have died as a result of severe reactions set up. Though the percentage of mortality was negligible, the severity of reactions set up in many cases could not be ignored though it was possible that such severity was due to an unusually high susceptibility of such reactors. The method proved to be unpopular among the ryots owing to the severe reactions set up, especially in buffaloes, and owing to a large percentage of animals becoming incapacitated for work for some time after inoculation. The possibility of setting up fresh foci of infection in healthy centres by this method had also to be considered.

Tissue virus

Exhaustive trials with infected goat spleen tissue were also carried out with a view to study the adaptability of this technique in this province and the results obtained have been published [Nair, Krishnamurti and Kalyanasundaram, 1941, 1942]. The adoption of the method of inoculation with tissue emulsion in normal saline was not found to be quite safe in this province, as the reactions set up after vaccination were rather severe. In the case of the desiccated vaccine, it was found to have set up severe reactions in some places and it could not be said to be a safe product.

It is the general experience that the administration of goat virus, either blood virus or tissue virus, which produces a reliable immunity, is attended with a considerable amount of risk and that a certain proportion of animals so inoculated, especially buffaloes, develop a severe reaction and in some cases mortality also occurs. As this method of protection with goat virus alone could not be carried out with safety, serum simultaneous inoculations is now being done using goat blood virus as the infective agent and reducing the quantity of protective serum. The serum given is just sufficient to ward off any undesirable reactions. Table IV shows the number of such serum simultaneous inoculations performed so far in this province and the mortality noted amongst them.

Rinderpest bull virus

The bull virus is maintained at this Institute by successive passages in young susceptible buffalo-calves similar to those used for testing goat virus.

TABLE IV

Number of 'serum-simultaneous method' of inoculations with rinderpest goat virus performed and the mortality amongst them

Years	Number of inoculations performed			Number of inoculated animals dying of the disease		
	In clean areas	In affected areas	Total inoculations	Among those that were inoculated in clean areas	Among those that were inoculated in affected areas	Total deaths
(1)	(2)	(3)	(4)	(5)	(6)	(7)
1937-38	5,197	36,907	42,104	..	8	8
1938-39	48,465	1,92,259	2,40,724	6	50	56
1939-40	40,944	2,54,597	2,95,541	..	102	102
1940-41	78,459	3,30,243	4,08,702	21	131	152
1941-42	74,027	3,30,527	4,04,554	21	133	154

The strain was obtained from one of the districts of this province, where the disease was prevailing. Bull virus was largely used for some years for conducting serum simultaneous inoculations but, now with the advent of goat virus, the former is mostly employed for carrying out immunity tests.

NATURE OF REACTIONS OBSERVED

An analysis of observations recorded for five years from 1938-39, leaving out of account the year 1942-43 when no bull virus strain could be maintained, is given in a tabular statement (Table V).

From the statement it may be observed that during the year 1938-39, 133 buffalo-calves were used for virus production; of these, though 130 calves had thermal reactions, only 102 were bled for virus, as the remaining 28 had not the satisfactory thermal rise to allow bleeding. One hundred and fifteen calves had diarrhœa and 72 had mouth lesions as well. There was a total mortality of 77 calves or 57·9 per cent including 17 which were not bled. During the year 1939-40, the percentage of mortality was 53·8, about 4 per cent less than in the previous year. During 1940-41 the mortality went down to 38·5 per cent. It remained constant at 42·3 per cent during 1941-42 and 1943-44. The higher percentage of mortality during the first two years may perhaps be attributed to greater quantities of blood drawn from the calves to meet the demand than that done during the subsequent years when there was not much demand for the product. Typical clinical symptoms were invariably seen in most of the reactors. High rise of temperature, varying from 103°F. to 106°F., with dullness, inappetence, suspension of rumination, discharge from eyes, followed by diarrhœa from about the sixth or seventh day, which in some cases was very severe with dysentery, congestion of buccal mucous membrane and, in some, ulcers also were noticed. Diarrhœa usually persisted till death in animals that died. Such animals were prostrate in condition and were found to be having shooting watery diarrhœa with offensive smell. In some dysentery was observed and it was so severe that they passed clots of blood and shreds of alimentary mucous membrane. In the case of animals that survived, diarrhœa was observed for about a week or ten days and the faeces gradually became normal. Congestion of buccal mucous membrane was usually observed from about the sixth or seventh day followed by necrosis and ulceration in many cases. Mild reactors had thermal rise for a few days, followed by passing of soft faeces for three or four days and then they returned to normal condition. On autopsy acute gastro-enteritis and occasional ulceration of abomasum, caecum, colon, etc. were observed.

COMPARISON OF RINDERPEST GOAT VIRUS WITH RINDERPEST BULL VIRUS

Taking into consideration that the same type of young susceptible buffalo-calves were used both for testing the virulence of goat virus and for production of bull virus, the reactions observed amongst them may comparatively be studied to give an idea of the virulence of both the goat and bull strains.

In the Mukteswar strain of goat virus, as already stated, it was observed that there had been the following percentage of reactions:

Total number of calves tested	139
Thermal reactions	116 or 83·5 per cent
Diarrhœa	86 or 61·9 per cent
Mouth lesions	39 or 28·1 per cent
Mortality	32 or 23 per cent

In the Madras strain, the following reactions were noted:

Total number of calves tested	42
Thermal reactions	40 or 95·2 per cent
Diarrhœa	31 or 73·8 per cent
Mouth lesions	16 or 38 per cent
Mortality	7 or 16·7 per cent

Totalling up the figures for both the strains, it may be said that out of 181 calves tested 156 or 86·2 per cent had thermal reactions, 117 or 64·6 per cent had diarrhœa and 55 or 30·4 per cent had mouth lesions also. There had been a mortality of 39 calves or 21·5 per cent among them.

It may be mentioned here that in our experiments with tissue virus in normal saline, there was also a mortality of about 20 per cent among the calves; it was almost the same as in the case of blood virus.

In the case of the calves used for bull virus production the percentages of reactions are noted below; the figures indicate totals for the five years already mentioned.

Out of a total of 479 calves used 418 or 87·3 per cent had thermal reactions, 324 or 67·6 per cent had diarrhœa and 182 or 38 per cent had mouth lesions and there was a mortality of 236 calves or 49·2 per cent amongst them.

The comparative figures furnished show that in the case of goat virus there is a decrease in the percentage of reactors by (1) about one per cent in thermal cases, (2) three per cent in diarrhœa cases, (3) 7·6 per cent in mouth lesions cases and (4) 27·7 per cent in mortality.

Thus, though there is not much difference in percentage of other reactions, the mortality in case of goat virus is much less, less than even half of the mortality noted in case of bull virus. It should be remembered that from the calves used for bull virus production, some quantity of blood is drawn for virus; but that may perhaps cause only a slight increase in the percentage of mortality.

Table V

Type of reaction observed among buffalo-calves used for bull virus production

Years	Number of calves used	Type of reactions observed: Thermal	Diarrhoea	Mouth lesions	Mortality	Number of non-reactors (those that had no reaction at all)	Number of calves bled	Mortality amongst the bled	Number of reactors not bled	Mortality amongst the non-bled (Reacted but not bled)
(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)	(11)
1938-39	133	130	115	72	77	1	102	59 *plus* 1 (destroyed for spleen)	30	17
1939-40	132	117	100	47	71	10	96	59	25	12
1940-41	65	55	35	22	25	10	47	19 *plus* 3 (destroyed for spleen)	8	3
1941-42	71	53	35	19	30	16	43	18 *plus* 8 (destroyed for spleen)	12	4
1943-44	78	63	30	22	33	11	56	17 *plus* 11 (destroyed for spleen)	11	5
Total	479	418 87·3 per cent	324 67·6 per cent	182 38 per cent	236 49·2 per cent	48	344	172 *plus* 23 (destroyed for spleen)	86	41

Total calves used	479
Number of calves bled	344
Number of calves reacting but not bled	86
Percentage of mortality amongst the bled	50 (excluding those destroyed for spleen)
Percentage of mortality amongst those reacting but not bled	46·5
Percentage of mortality amongst the total number of calves used	49·2

Summary

A brief survey of the work done on the goat virus alone method of inoculation in the Madras Province is given.

Two strains of goat virus, the Mukteswar strain and the Madras strain, are maintained at the Serum Institute, Madras, and there is not much difference in the nature and percentage of reactions set up by them in the control calves.

It was found both from experiments conducted in the laboratory and in the field that the method of protecting cattle against rinderpest by the goat virus alone was not quite a safe one. The reactions set up by this method in the control calves were severe resulting in a mortality of 21·5 per cent amongst them. In the field, though the percentage of mortality was negligible, the severity of reactions set up by this method, especially in buffaloes, was such that it became unpopular among the ryots who could ill-afford to have some of their animals incapacitated for work and their milk yield reduced even for a short period.

The spleen tissue emulsion in normal saline solution also behaved in the same manner as the blood virus; hence it could not also be advocated for use without anti-serum.

From experience gained in this province it may be said that the ideal attenuation of the virus for use as such without anti-serum, in bovines, cannot be said to have been obtained by successive passages through goats.

The percentage of mortality among goat virus control calves was observed to be much less, less than even half of that which occurred among the calves used for the production of bull virus, though appreciable difference was not noticed in the degree and nature of other reactions.

Acknowledgements

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IMMUNIZATION OF BUFFALOES AGAINST RINDERPEST IN ASSAM*

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Rinderpest in buffaloes is of common occurrence in Assam and heavy mortality is often observed. The technique of goat virus vaccination, with or without serum, has been adopted for the control of outbreaks. The object of this paper is to present a brief description of certain features observed in the course of immunization of the local types of buffaloes and those in different localities of the province. It may be stated that vaccination with goat tissue virus (1 c.c. of 1 : 200 spleen emulsion in saline), has been adopted as a routine, both as a prophylactic and for control of outbreaks in cattle and this method has given very encouraging results for some years now.

In regard to buffaloes, immunization is usually undertaken in actual outbreaks of rinderpest and not as a prophylactic measure. This is mainly due to the fact that there is evidence of a wide range in the degree of reaction in the local types of buffaloes and also in buffaloes in different areas. A standard routine for vaccination of buffaloes could not be fixed as the dosage of serum is variable and has to be regulated in accordance with these two factors. Field observations indicate that the reaction is more severe in buffaloes than in cattle. As a result of such severe reaction, partial reduction or complete cessation of milk yield and diarrhœa have been observed ; even deaths among the vaccinated have occurred. Such disquieting factors are likely to affect adversely the popularity of vaccination.

Buffaloes are maintained generally for milk and they are in some respects more economical milk producers than either indigenous or crossbred cattle. Besides, there appears to be a tendency, though not a very marked one, to displace bullocks for traction purposes by male buffaloes, especially in ploughing in marshy lands. At present the male stock is of great utility in certain types of cultivation and, as such, the buffalo has an important place in the general agriculture of the province. Also the poor quality of Assam cattle is in marked contrast to the fine quality of the Assam buffalo. Hence a fairly large number of buffaloes is maintained in Assam and the opportunity was taken to immunize them with the least mishap, as far as possible, by exercising great caution.

Types of buffaloes and their localities

A brief description of the types of buffaloes in Assam would be helpful to ascertain the degree of their susceptibility. It can hardly be claimed that this province has any recognized breed of buffaloes. However, they may be roughly classified as under :

(1) *Local or Assam buffaloes.* These are massive and fierce animals ; sometimes there is very little difference between this type and wild buffaloes.

(2) *Local hill buffaloes.* These are mainly Manipuri buffaloes and, to a less extent, those found at the foot-hills of Mikir, Khasi and Jaintia Hills, etc., and are generally smaller in size.

(3) *Imported buffaloes or Bangar.* These are imported mostly from Bihar and also from Bengal. The animals are much quieter in temperament and can be recognized from the indigenous types by their medium size.

Although it may be possible to distinguish the above types with a fair degree of accuracy, a variety of crossbred animals have developed due to indiscriminate admixture of blood from all these types. Further, the quality of buffaloes in Assam is said to be kept up by crossing with the wild buffaloes. A wild buffalo frequently attaches himself to a herd and is usually tolerated by the herdsmen [Blackwood, 1916].

Regarding localities, it may be stated that buffaloes are distributed both in the plains and hills of Assam. In the plains of the Assam valleys, buffaloes of local, imported and mixed breeds are found. Where the plains border on the hill region, hill buffaloes and their mixed breeds are common. Buffaloes in the Surma valley are mostly of mixed breeds of Manipuri and Bangar and pure Manipuri. Apart from the Assam and Surma valleys, the rest of the province consists of ranges of hills. Starting on the west of the Assam range, we have in succession the Garo Hills and the Khasi and Jaintia Hills which are joined by North Cachar to the Naga Hills on the north-east and to Manipur on the south-east. South-west of Manipur are the Lushai Hills. The average elevation is about 4,000 ft but some peaks rise to 7,000 ft [McSwiney, 1912]. Buffaloes in the lower slopes of the hills, that approach the plains, are mainly local hill type and to a less extent mixed breeds.

Immunization

It would appear necessary to describe the method of immunization in each circle in the Assam Valley where mixed herds of varying susceptibility are usually met with. In the Lower Assam Circle the local buffaloes are vaccinated with 0·5 c.c.—that is, half the normal dose—of goat tissue vaccine alone. The reaction is mild with slight reduction

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in milk yield, in some cases even by one-half. They recover in the course of a fortnight and mortality is very rare. Animals of mixed breed (local and hill types) in this locality are given 0·5 c.c. of vaccine and a flat dose of 40 c.c. of ordinary anti-rinderpest serum simultaneously. The reaction manifested in several cases is mild with slight decrease in milk yield; a few develop diarrhœa and exhibit a marked reduction in milk yield. Very rarely, a few deaths have been reported (maximum mortality, one per cent); these were probably attributable to the fact that they were not properly cared for during the reaction period or to helminthic infestation.

In the Central Assam Circle, the general procedure is to vaccinate the buffaloes by using 0·5 c.c. of vaccine and a flat dose of 20 c.c. of serum. The animals show moderate reaction with mild clinical symptoms and slight reduction of milk and recover in about a fortnight.

Local buffaloes in the Upper Assam Circle are immunized by injecting 0·5 c.c. of vaccine and 30 to 60 c.c. of serum. Buffaloes of mixed breed, generally of local and imported types, are given the same dose of vaccine and 20 to 50 c.c. of serum. Comparatively, the reaction is more manifest in the local than in the mixed breed. Rise of temperature of about 2 to 3°F. may be noted. Reduction in milk is observed from the fourth or fifth day up to about two to three weeks. Diarrhœa is noticed in some cases. In Sibsagar, 487 buffaloes have been vaccinated since 1939-40. It is of interest to note that no death was reported in the vaccinated animals and also there was no outbreak of rinderpest among them till 1942-43.

In the Surma valley, most of the buffaloes consist of a mixed breed of Manipuri and Bangar and of pure Manipuri. The following methods of vaccination have been tried:

(i) Dose of 0·5 c.c. of G.T.V. (1 : 200) and 30 to 40 c.c. of ordinary rinderpest serum was given in actual outbreaks and also for prophylaxis. 190 animals were vaccinated.

(ii) Dose of 0·5 c.c. of G.T.V. alone was used as a prophylactic measure in 388 buffaloes.

(iii) Dose of 0·25 c.c. of G.T.V. alone was given as a prophylactic measure in 184 buffaloes.

In the first method the reaction was mild; the course was not much prolonged but appeared within normal limits; slight reduction in the milk yield was also noticed. All the vaccinated animals recovered satisfactorily. In the second method the reaction was found to be very severe in about 50 per cent of cases. High temperature, marked reduction in milk-yield, diarrhœa and dysentery were observed. The symptoms were really alarming in a few cases, and of these three buffaloes died. The incubation period appeared rather shorter (thermal reaction occurred on the third day), the course was prolonged and the convalescence slower. In the third method there was not much difference in reaction as compared with the second one. The incubation period was found to be a little longer (thermal reaction occurred on 4th to 6th day), but the severity of reaction was almost the same, resulting in two deaths. It would appear that for Manipuri buffaloes, both pure and mixed, the safe and effective method of immunization would be to use 0·5 c.c. of vaccine and 30 to 40 c.c. of ordinary anti-rinderpest serum simultaneously. In the 'vaccine alone' method, it seems probable that a higher dose of vaccine would tend to reduce the period of incubation a little. The use of serum—at least 30 to 40 c.c.—is essential to control severe reactions which otherwise would entail mortality.

In some cases disturbance in health due to helminthic infestation is observed as a result of vaccination, i.e. about 10 to 15 days following vaccination. Diarrhœic motions are passed and there is a rapid fall in condition. Anaemic changes in the blood and fairly large numbers of ova of *Distoma*, *Amphistome*, *Strongylus*, etc., are noticed. Parasitic infestation in buffaloes in this province is very common and fairly heavy. This is inevitable due to the climatic and geographical conditions of Assam. Such infestations flare up sometimes following the reaction of rinderpest vaccination.

Discussion

It will be observed that the method of immunization of buffaloes against rinderpest in Assam requires a careful interpretation of the dosage of serum, depending upon the locality and the type of breed. The reason is that the buffaloes possess varying degrees of susceptibility as they consist of hill, wild or half-wild and imported types and their crossbred offsprings. It has been found that one method, say 0·5 c.c. of vaccine alone, however satisfactory in a particular locality, turns out to be a highly risky one in another area. Mention should also be made of some cases in which the reaction is uncertain. Hence, for immunization of buffaloes with goat tissue vaccine, it is now considered essential that a dose of serum be given to ward off any likelihood of risk.

A perusal of the reports of the provinces and states in India indicates that virus-cum-serum method is efficacious in the immunization of buffaloes. In several provinces goat tissue virus is used with serum simultaneously, while in Mysore the method of goat blood virus with serum is preferred. [Achar, 1941]. However, in some areas in the Punjab, it is reported that buffaloes are vaccinated with goat tissue virus alone without untoward results [Chowdhury, 1941].

Reduction in milk-yield is perhaps one of the most disturbing consequences of vaccination, causing

anxiety to the owner and the operator alike. The most satisfactory method appears to be the use of adequate quantity of serum which inhibits severe reaction and thus causes a minimum loss in milk yield. Such loss is inevitable as inoculation, operation or even any physical interference with the buffaloes affects these temperamentally and usually reduces their milk-yield for one or two days. It may, however, be mentioned in this connection that in the Central Provinces and Berar the method of blood virus-cum-serum was experimentally found to be the safest; the loss in milk yield being lowest, viz. 25 per cent [Singh, 1939].

Fall in condition or general health has also been observed following vaccination in the field. Although severe reaction is likely to bring about this condition, the absence of green fodder aggravates it. It would be preferable as far as possible to undertake vaccination as far as at a time when there is ample green fodder.

Summary

A brief description of the types of buffaloes in Assam and their distribution in different localities, is given.

Rinderpest is of common occurrence in Assam, causing heavy mortality among buffaloes. Details are given of the field experiments conducted to evolve the most suitable method of immunization of buffaloes against the disease in the province.

Particulars of the type and degree of reaction in the methods adopted to control the disease are described. There is evidence of a wide variation in the degree of reaction in the local types of buffaloes and also in buffaloes in different areas, viz. plains, hills and forests.

Vaccination with goat-tissue virus, along with 30 to 40 c.c. of serum, appears to be the safest method, as it obviates to a large extent the risk of mortality and loss in milk yield.

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STREPTOCOCCUS MASTITIS IN BUFFALOES

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IT has been generally assumed locally that mastitis in buffaloes is of little importance. Since relatively slight experience convinced us that this view was incorrect, work on the problem was begun with the results outlined below.

It was soon realized that the examination of several hundred milk samples by a plating technique was beyond the capacity of this laboratory. It was therefore decided to adopt the incubated milk technique described fully by van Rensburgh [1941] and since reported with some favour by Rowlands and Field [1943] and by Field and Smith [1944]. Briefly, this technique consists of incubating mixed quarter samples of milk from each animal at 37°C. and examining stained smears for the presence of streptococci 18 to 24 hours later.

No attempt has been made here to compare the van Rensburgh technique with any other, but it is worth mentioning that, when occasion has demanded the isolation of mastitis streptococci, these organisms have invariably been isolated from van Rensburgh positive samples.

Our work is recorded under the three headings, general epidemiology, vaccination and eradication.

General epidemiology

Bacteriology

The vast majority of infected buffaloes examined by us have been infected with streptococci and this work has been concerned entirely with that type of organism. The number of samples containing staphylococci has been relatively low, although a certain number of clinical cases have been associated with *Staph. aureus*. The number of *C. pyogenes* cases has been even lower, though this organism too has been isolated occasionally. No case of tuberculous mastitis in buffaloes has been met with, but serious search for this organism has not been made.

We have not carried out serological tests in this laboratory on mastitis streptococci but, judging by the biochemical reactions, they would nearly always fall into Lancefield's group B, as might be expected. These local strains give β-haemolysis in horse-blood agar, a final pH in glucose-broth of about 4·3 after four days incubation, hydrolyse sodium hippurate, fail to reduce 1 in 5000 methylene blue in milk, produce acid in lactose and trehalose,

sometimes in salicin, but not as a rule in mannitol or sorbitol.

General incidence

Before any policy concerning mastitis could be suggested, it was necessary to be convinced that the disease was in fact widespread and of importance. Between May and October, 1944, a preliminary survey was therefore undertaken on three different farms; during the same period, another laboratory working in cooperation with us undertook the examination of two more farms.* The results are recorded in Table I from which it may be seen that the incidence of infection varied from 13·1 to 18·5 per cent, and that of the total number of 3,322 animals examined 16·0 per cent were infected. Since the original survey, other examinations have been carried out on some of these farms and the results have confirmed the finding of a fairly high incidence of infection.

The indications were therefore that streptococcus mastitis was fairly widespread and it was to be assumed that it was the cause of considerable economic loss.

TABLE I

Incidence of streptococcus mastitis in buffaloes in Lahore area.

Farm No.	Number examined	Positive
1	511	93 (18·2 per cent)
2	1006	168 (16·7 per cent)
3	625	90 (14·4 per cent)
4	694	91 (13·1 per cent)
5	486	90 (18·5 per cent)
TOTAL	3322	532 (16·0 per cent)

Possible intermittency of excretion of streptococci

The original estimate of approximately 16·0 per cent infection was based on the results of one test only. A point of considerable importance however in dealing with this disease is the fact that there is in buffaloes an apparent intermittency of excretion of streptococci. This is shown in Table II. Of the 730 animals examined, twice, with an interval of about three months between tests, 122 were positive at the first test and of these only 60 (49·2 per cent) were positive at the second test. Similarly of the 608 negative at the first test 106 were positive at the second.

TABLE II

Apparent intermittency of excretion of streptococci at consecutive tests

—		Second test		TOTAL
		Positive	Negative	
First test	Positive	60	62	122
	Negative	106	502	608
TOTAL		166	564	730

This point needs more thorough examination. It may indicate limitations of technique; it may be due to contaminants preventing growth of streptococci, or there may be a true intermittency of excretion. In the meantime it may be said that our figures do not suggest any relationship between intermittency of excretion and age or period of lactation.

Association of mastitis with age

In general it has been the finding in cattle that the older the animal the more likely it is to have mastitis. This would seem a reasonable *a priori* hypothesis; other factors being equal there must be an increasing incidence with increasing exposure. That this is also the case in buffaloes is shown by the results recorded in Table III. From this table it may be seen that the percentage of positive animals increases from 12·5 among animals 4 to 6 years old up to 30 among animals over 11 years old.

TABLE III

Increase of mastitis with advancing age

Age in years	Number of animals examined	Percentage infected
4-6	24	12·5
7	83	13·3
8	202	19·8
9	258	20·2
10	249	23·7
11	157	25·5
12 and over	60	30·0

* For permission to publish the results from Farms 4 and 5 we are indebted to Mr Abdul Qayum Khan of the Dairy Bacteriology Laboratory, Lahore.

Association of mastitis with period of lactation

It has been suggested that the early stages of lactation constitute the period of greatest susceptibility to mastitis in cattle. Our results indicate a similar finding in buffaloes, though our numbers were too small to be statistically significant. It may be briefly stated that at all ages up to 10 years there was a greater incidence of mastitis during the first hundred days of lactation than at later stages. Summarizing the data it was found that of the 479 animals examined during the first hundred days of lactation 16·1 per cent were infected, and of the 302 examined during the second hundred days 10·9 per cent were infected.

Milk yields of infected and non-infected buffaloes

It is accepted that cows give a reduced milk yield when they become infected with mastitis streptococci but very few workers have actually demonstrated this with figures. Minett and Martin [1936] studied by statistical methods the relationship of milk yield to infection by *Br. abortus* and *Str. agalactiæ*. In the case of the latter they found that with Ayrshire cows in one herd the corrected yield was reduced by 10·8 per cent, with Friesian cows in two herds the percentage reductions in corrected yields amounted to 16·5 and 19·5.

We obtained milk yield data for a small number of buffaloes seven years old and over. The animals were examined for mastitis streptococci in October 1944, and again in January 1945. The yields for October, November and December, 1944, were tabulated according to the age of the animals and the stage of lactation at the time of the first test. Only animals which were negative at both tests or positive at both tests were included. The results indicated that the yield, as would be expected, increased as lactation advanced, at any rate up to 200 days.

TABLE IV

Average milk yield (in lb.) over three months of infected and non-infected 7-11 years old buffaloes

Buffaloes	Period of lactation (in days) during which the first test was carried out			
	1-50	51-100	101-150	151-200
Non-infected . .	1586 (89)	2090 (58)	2371 (51)	2989 (30)
Infected . . .	1151 (21)	2059 (15)	1197 (11)	926 (4)

Figures in brackets represent numbers of animals from which averages have been calculated.

In Table IV are summarized the results for 7 to 11 years old animals; figures for animals over 11 years old are not included since there were so few of them available. From the table it may be seen that among non-infected animals the average total milk yield in lb. for the three months increased from 1,586 in early lactation to 2,989 later; among infected animals, at least after they had been in lactation over 100 days, there was a decrease in milk yield, which might have been brought about by progressive induration of the udder. Again, it is to be noted that at all stages of lactation the infected animals have given less milk than non-infected animals. We think that these results, perhaps inconclusive by themselves, lend support to the general belief that mastitis infection leads to a lowering of milk yield.

Vaccination

In September 1944, there was an opportunity to carry out a controlled vaccination experiment. While we did not expect vaccination to be beneficial, the opportunity seemed too good to miss and experimental work was therefore commenced. Vaccine was prepared from five strains of streptococci isolated from buffaloes in the herd concerned. All strains gave β-hæmolysis in deep horse-blood agar. Two strains were long-chained, apparently typical *Str. agalactiæ*, while two others formed relatively short chains, fermenting lactose, trehalose and salicin but not mannite or sorbite. The fifth strain was short-chained and fermented all five sugars; this strain in addition, contrary to the other four, reduced 1 in 5,000 methylene blue in milk. The organisms were grown for 48 hours on Fildes' agar and then washed off with sterile saline, the thick suspension being heated at 56°C. for four hours. Subcultures made after this time were always sterile. Enough 10 per cent phenol was then added to give a final concentration of 0·5 per cent and the suspension was stored in the ice-chest until required. A few days before use, the suspension was diluted with 0·5 per cent carbol-saline to the required density.

Initial test of the vaccine

Since a relatively large number of animals had to be vaccinated, it was essential to be certain that the vaccine had no marked deleterious effect on milk yield. Ten buffaloes were therefore inoculated subcutaneously with 5·0 c.c. suspension, standardized to No. 2 of the Wellcome Opacity Tubes (approximately 600×10^6 organisms per c.c.). Seven days later these animals received a second dose of 5·0 c.c. ten times as strong (i.e. twice as dense as tube 10 of the standards). Milk yields were recorded daily from seven days before the first inoculation to seven days after the second inoculation.

The total weekly yield in lb. for each of the animals is shown in Table V. It may be seen that the total yield of the ten animals for the week preceding vaccination was 699 lb., for the week following the first inoculation 680 lb., and for the week after the second inoculation 732 lb. From these results it was concluded that vaccination would have no appreciable effect on milk yield.

TABLE V

Effect of two subcutaneous inoculations of streptococcus vaccine on the milk yield of buffaloes

Buffalo No.	Milk yield (in lb.) First week		Milk yield (in lb.) Second week		Milk yield (in lb.) Third week
1	81	First dose of vaccine inoculated	71	Second dose of vaccine inoculated	98
2	54		53		42
3	90		87		113
4	43		43		36
5	63		72		72
6	71		63		56
7	73		86		90
8	50		32		39
9	91		98		112
10	83		75		74
Total yield .	699		680		732

Vaccination of non-infected animals

One hundred and fifteen non-infected animals kept on Farm 1 were available for experiment; 52 of these, selected purely at random, were vaccinated and 63 were left as controls. The vaccinated animals received two doses of vaccine subcutaneously at an interval of seven days. The first dose consisted of 5·0 c.c. suspension standardized to No. 1 of the Wellcome Opacity Tubes and the second of 5·0 c.c. suspension ten times as dense, i.e. the doses were half those used in the initial test of the vaccine.

A day or two prior to vaccination milk samples from all animals were examined for streptococci. Approximately four months after vaccination milk samples were again examined. The results are recorded in Table VI from which it may be seen that nine (17·3 per cent) of the vaccinated animals and eight (12·7 per cent) of the controls had become infected. The difference of 4·6 per cent in favour of the controls is not statistically significant since it is only 0·7 times its standard deviation.

It may safely be concluded therefore that vaccination had been without effect as a means of preventing streptococcal mastitis.

TABLE VI

Efficacy of vaccination as a means of preventing streptococcus mastitis in buffaloes

—	Positive	Negative	Total
Vaccinated . .	9 (17·3 per cent)	43	52
Control . .	8 (12·7 per cent)	55	63
TOTALS .	17	98	115

Difference=4·6 per cent S.E.=6·6

Vaccination of infected animals

In addition to the 115 non-infected animals, there were also available 38 infected animals; of these, 17 were vaccinated and 21 were left as controls. From Table VII it can be seen that 10 (58·8 per cent) of the vaccinated and 10 (47·6 per cent) of the control animals were negative four months later. The difference of 11·2 per cent in favour of the vaccinated is not statistically significant. It may be noted incidentally that the finding that about 50·0 per cent of animals positive at one test are negative at a subsequent test is in accordance with results obtained previously.

TABLE VII

Efficacy of vaccination as a means of curing streptococcus mastitis

—	Positive	Negative	Total
Vaccinated .	7	10 (58·8 per cent)	17
Control . .	11	10 (47·6 per cent)	21
TOTALS .	18	20	38

Difference=11·2 per cent S.E.=16·3

It was concluded from this experiment that vaccination had not led to any cure.

Eradication

Anticipating that vaccination would be useless, experiments on eradication were carried out on farms 1 and 3 concurrently. The experiments were designed to test the efficacy of partial eradication and also that of a more thorough eradication.

Partial eradication

On Farm 1 there were six sheds of animals. Three of these were already in the vaccination experiment.

In September 1944, the three remaining sheds contained 100, 100 and 97 animals; the initial numbers of infected animals were 27, 13 and 17 respectively as judged by a single milk examination. In the last shed the 17 infected animals were moved to one end; they were milked and attended by two *gawalas*, one incharge of 13 infected animals and the other incharge of the remaining four infected together with nine clean animals. In the first two sheds no movement of animals took place, and they were in fact regarded as controls. In view of the fact that vaccination of animals in the other three sheds had no effect, these animals may also be regarded as controls. In other words, in September 1944, there was one shed with infected animals at one end and five sheds with infected animals distributed throughout the length of the buildings. The arrangements on this farm were not very satisfactory and cooperation was only half-hearted. No milk examinations were carried out on animals entering the herd, hence infection had every chance of being introduced.

Four months later all animals were again examined and the results assessed. Of the 221 originally non-infected control animals, 39 or 17·6 per cent had become infected; of the 32 originally non-infected animals in the partially eradicated shed, five or 15·6 per cent had become infected. The difference of 2·0 per cent is clearly not significant.

It seems from this small experiment that incomplete methods of eradication cannot be expected to cope successfully with the spread of mastitis.

Complete eradication

Farm 3 contained 11 sheds of milking buffaloes. In October 1944, when a complete examination of the whole herd was carried out, the original distribution of animals was as shown in Table VIII. Thus shed 1 had 57 animals of which nine were infected, shed 2 had 57 animals of which five were infected and so on. On this farm the management was very much more cooperative and experimental conditions were more favourable.

A day or so after the first test it was decided to eradicate all infected and doubtful animals from sheds 3, 4, 5 and 8 and replace them with negative animals from other sheds. These four sheds were selected because they were in a position on the farm where drainage and passage of milkers was *away from* and *not through* them. The actual transfer was carried out as follows: from shed 3, four doubtful and seven infected animals were removed to shed 10 and replaced by 11 negative animals from shed 10; from shed 4, one doubtful and five infected animals were removed to shed 10 and replaced by six negative animals from this shed; from shed 5, seven doubtful and five positive animals were removed to shed 11 and replaced by 12 negative animals from shed 11; and from shed 8, three doubtful and nine infected animals were removed to shed 11 and replaced by 12 negative animals from this shed. The distribution after this move is shown in Table VIII.

TABLE VIII

Effect of eradication on the distribution of mastitis in buffaloes

Shed No.	Original distribution		Distribution after first test in October 1944		Distribution in January 1945		—
	Number of animals	Positive	Number of animals	Positive	Number of animals	Positive	
1	57	9 (15·8 per cent)	57	9 (15·8 per cent)	55	16 (29·1 per cent)	
2	57	5 (8·8 per cent)	57	5 (8·8 per cent)	47	7 (15·6 per cent)	Control sheds
6	51	11 (21·6 per cent)	51	11 (21·6 per cent)	54	11 (20·4 per cent)	Control sheds
7	54	11 (20·4 per cent)	54	11 (20·4 per cent)	53	15 (28·3 per cent)	Control sheds
9	56	7 (12·5 per cent)	56	7 (12·5 per cent)	55	18 (32·7 per cent)	Control sheds
10	56	11 (19·6 per cent)	55	23 (41·8 per cent)	51	31 (60·8 per cent)	Control sheds
11	57	10 (17·5 per cent)	57	24 (42·1 per cent)	49	24 (49·0 per cent)	Control sheds
3	58	7 (12·1 per cent)	58	..	40	6 (15·0 per cent)	Eradication sheds
4	59	5 (9·3 per cent)	59	..	46	7 (15·2 per cent)	Eradication sheds
5	54	5 (9·3 per cent)	55	..	56	4 (7·1 per cent)	Eradication sheds
8	56	9 (16·1 per cent)	56	..	43	8 (18·6 per cent)	Eradication sheds

From this time onwards for the next three months milk samples were examined from all animals prior to their entry into the milking herd. Only animals with negative milk samples were allowed into the four eradication sheds. In this way it was hoped to exclude infection. Owing to the assumed intermittency of excretion of streptococci it was considered inevitable that a few infected animals would slip through, especially as only one test was carried out prior to entry into the herd, and this a day or two after calving. Actually, owing to an administrative error, one known infected animal was admitted to shed 8 about one month before the completion of the experiment.

In January 1945, the whole herd was again examined with the results shown in Table VIII. From this table it is clear that after three months the eradication sheds had considerably fewer infected animals than the control sheds; thus in the eradication sheds there were 185 animals of which 25 or 13·3 per cent were infected, whereas among the 364 controls 122 or 33·5 per cent of the animals were infected. In view of the fact that sheds 9, 10 and 11 were repositories for infected animals the comparison of the eradication sheds with the grand total of the controls is perhaps not a fair one. However if only sheds 1, 2, 6 and 7 of the controls are considered there will still be found to be a 10 per cent difference, since among the 209 animals in these sheds 49 or 23·4 per cent were infected.

Table IX

Spread of mastitis among originally negative buffaloes

Shed No.	Numbers of negative animals in October 1944	Number of positive in January 1945	Percentage positive in January 1945
1	43	8	10·2
2	19	1	
6	36	1	
7	39	4	
9	42	13	36·8
10	29	14	
11	24	8	
3	26	3	9·8
4	25	3	
5	46	3	
8	26	3	

If the observations are confined to animals negative at the first test which remained in the herd for the whole period of three months, i.e. excluding all new entries, an idea of the actual spread of mastitis under different conditions will be obtained. In Table IX we give the results of the examination of the 355 animals concerned. In sheds 1, 2, 6 and 7 among 137 originally negative animals there were 14 or roughly 10 per cent new infections within the three months; similarly in sheds 3, 4, 5 and 8 of the 123 originally negative animals about 10 per cent became infected. The removal of infected animals had not therefore appreciably effected the normal spread of the disease. In sheds 9, 10 and 11 where the initial infection rate was much higher (over 30 per cent) and a constant supply of infection was maintained, the spread to negative animals was much more pronounced; of the 95 originally negative animals 35 or 36·8 per cent became infected.

Discussion

The data presented in this paper suggest that streptococcus infection of the buffalo udder is a good deal more prevalent than is generally suspected. It would seem that infection is spread primarily by the milkers. It is the practice here for one *gawala* to attend to and milk about twelve or thirteen animals. When infection appears, it arises in groups of animals standing in adjacent standings; as a rule these foci of infection are *within* a particular *gawala's* domain and do not overlap to the next one.

A factor which may be of prime importance in treating or eliminating mastitis is the intermittency of excretion of streptococci; this is perhaps especially the case in buffaloes. It is clear that a single test of the van Rensburgh type will not be sufficient to pick out all infected animals. Referring to Table II, for instance, it may be seen that of the 564 animals negative at the second test, 62 (11·0 per cent) were in fact known to be infected from the result of a previous test. This is a serious difficulty and its only solution at the moment lies in repeated examinations of negative animals.

In spite of the many difficulties our results tend to show that mastitis control in buffaloes is a practical and probably an economic proposition. By relatively simple methods, we have managed to limit the spread of the disease for the short period that the animals were under our control. Had repeated tests been carried out on the negative animals, we could presumably have reduced the incidence of the disease still more.

In view of the present and likely future position of the buffalo as a milk-producing animal in this country, the most important feature brought out by the work recorded here is the simplicity of the technique described as a means of diagnosing streptococcus infection. In this country, at any rate

during the summer months, given a few slides and a supply of Newman's stain diagnosis can be made by anyone proficient in the use of a microscope. Even if trained microscopists are not available, the difficulties will not be overwhelming. One of the difficulties in dealing with mastitis in the past has been the lack of laboratory facilities. Milk samples reaching a laboratory from any great distance naturally arrive in a putrid state due to overgrowth of contaminating organisms, unless steps are taken to prevent this. The deep blood agar plating technique has been a failure under such conditions. Now, with the incubated milk technique available, the solution is obvious. Samples can be collected in sterile tubes and incubated near their source; in the summer months in the greater part of India incubation will be unnecessary, as samples can be left standing at room temperature for the requisite time. After 18 to 24 hours smears can be made and forwarded to a laboratory when and as convenient; or if necessary a few drops of formalin or other preservative can be added to the milk samples and the whole tube sent for examination; any streptococci present would by then have grown out and would remain unaffected by the preservative while the preservative itself will prevent any further growth of outside contaminants. This technique is not only simple but quick; as an example, it may be noted that in this laboratory on one occasion two workers prepared, stained and examined 561 smears in one day (under eight hours); incidentally all the smears were examined by one man. If smears are already prepared and sent for diagnosis one small laboratory can therefore quite easily deal with several thousand specimens a week.

We feel that this is an important observation which needs emphasis, particularly in areas from which laboratory facilities are distant. If any mastitis control has been considered in the past, the policy adopted or suggested must have been influenced by the then great practical difficulty of diagnosis. This difficulty has now been removed and the policy can be modified accordingly.

SUMMARY

1. Examination of 3,322 milk buffaloes on five different farms by the technique of incubated milk samples revealed an average of 16·0 per cent streptococcus infection.

2. Infected buffaloes apparently excrete streptococci intermittently. As many as 50 per cent known infected animals may be negative at a given test.

3. Additional evidence is brought in favour of the general belief that the incidence of mastitis increases with the age of the animal, and that the early period of lactation is the period of greatest susceptibility.

4. Some evidence is adduced in favour of the belief that non-infected animals give a higher milk yield than do infected ones.

5. In our hands vaccination as a means of preventing or of curing the disease has been quite without effect.

6. Eradication by the removal of infected animals is indicated as a practical proposition. To be successful strict adherence to commonsense principles must be enforced; any relaxation of these principles is likely to lead to disaster.

7. It has been noted that the spread of the disease is materially affected by the number of infected animals present, at least when these are above a certain minimum.

8. The simplicity of the technique described by van Rensburgh and its possible role in a national policy of mastitis eradication are emphasized.

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FLUORESCENCE IN *GHEE* AND DETECTION OF ITS ADULTERATION

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THE last 25 years have seen a remarkable increase in the literature of fluorescence analysis in ultraviolet light. Dairy products have been examined in some detail—butter in the West [Haitinger Grengros and Schultz, 1927], and its counterpart *ghee* in India. Jha [1939] and Muthanna and

ukherji [1940] suggested that adulteration of *ee* up to 10 per cent was detectable; but Narasimamurthy and Suryanarayanamurthy [1940] found onflicting fluorescence colours for *ghee* and were opinion that the method offered no hope.

It was thought profitable to make a rather more xtended study than the previous workers and take nto account a few at least of the numerous factors hat complicate any examination of a physiological product like *ghee*. The practical aim was to see if t was possible to detect easily the adulteration of *ghee* with foreign fats. For this reason, the test was kept qualitative only.

A Hanovia, ∩-shaped mercury arc was used as the source of light, and a uviol filter used to cut off all but ultra-violet light. The samples were examined in thin tubes of non-fluorescent glass in a dark room. Observations were made at room temperature (23°-26°C.). The results are arranged to facilitate discussion.

TABLE I

Milk and milk products

Sample	Colour by day light	Colour by ultra violet light
Buffalo or cow milk	White	Bright yellow
Butter	White solid, colourless liquid	Yellow when solid, greenish yellow on melting
Buttermilk	White granular	Dull yellow
Curd	Thick white semi-solid	Dull yellow

The yellow colour of these products could be expected to be present in *ghee*, if at all, in very minute quantities only and therefore, would not interfere, to any appreciable extent, with the fluorescence colour of *ghee*.

TABLE II

Adulterants of ghee *commonly used*

Sample	Colour by day light	Colour by ultra-violet light
Crude cocoanut oil	Pale yellow	Fairly dark blue
Crude groundnut oil	Pale greenish yellow	Bright purple
Groundnut hardened oil	Colourless semi-solid and liquid	Very bright purple in both semi-solid and liquid states
Cocoanut hardened oil	do.	do.
Cotton seed hardened oil	Colourless waxy solid, melting to colourless liquid	Pale blue when solid; bright purple on melting

All these fluoresce various shades of blue and this is an encouraging feature from the point of view of the analyst.

Some workers, e.g. Musher and Willoughby [1929] state that the method of expression or extraction of an oil has some effect on its fluorescence. To see if this was the case, a deeper study was made with groundnut oil which was easily available. The results show that the difference is only one of degree and, as such, has no bearing on the qualitative detection of adulteration.

TABLE III

A study of groundnut oil

Sample	Colour by day light	Colour by ultra-violet light
Cold drawn processed oil	Pale yellowish green	Bright purple
Heated and drawn oil	Light yellow	Pale bluish violet
Refined and bleached oil	Very pale yellow	Dark sky blue
Unrefined hydrogenated oil	Colourless both in the solid and molten state	Pale lilac when solid; dark violet on melting
Finished, deodorized, hardened oil	Colourless solid and liquid	Light blue when solid; dark blue on melting

In Table IV are recorded the fluorescence colours of some factors that might possibly enter into the fluorescence colour shown by *ghee*.

Lustig and Botstiber, and Niethammer as quoted by Radley and Grant [1938] record a pale yellow colour for butyric acid and a deeper purple for oleic, probably because purer samples were used. The free acids could therefore be expected to give a blue colour taken together. The free acids extracted from a rancid sample of *ghee* (amounting to 4·5 per cent expressed as oleic acid) by neutralization, removal of the soap and subsequent liberation of the acids, gave on examination a beautiful purple colour.

Carotene gives an intense yellow colour; and chlorophyll extracted from leaves by the method of Willstatter and Stoll [1928] a ruby-red colour. The latter however would be an insignificant constituent of *ghee* if at all. Annatto does not fluoresce, but the dirty red colour it imparts to *ghee* is easily removed with animal charcoal. Lactochrome solution was obtained by Würsters' modified method [Radley, 1933] from whey; it is an important constituent of fresh *ghee*, but is easily destroyed on storage.

It has been suggested that the unsaponifiable matter from *ghee* and from oils might fluoresce differently. The results, however, were uniformly disappointing. The unsaponifiable matter was

TABLE IV

Constituents of ghee *and milk, colouring matters, breakdown products, etc.*

Sample	Colour by day light	Colour by ultra-violet light
Acids		
Acetic	Colourless liquid	Colourless liquid
Butyric	do.	Bright purple
Stearic	Colourless solid	Beautiful violet
Oleic	Pale, orange yellow liquid	Pale purple
Lactic	Colourless liquid	Pale lilac
Other possible constituents		
Alcohol	Colourless liquid	Very pale blue
Glycerine	Colourless viscous liquid	Extremely faint blue
Cholesterol	White, crystalline flakes	Bright purple colour
Lactose	Colourless solid	Pale blue colour
Natural and artificial colouring matters		
Carotene solution in petrol ether	Pale yellow in very dilute solution; orange residue on evaporation	Bright milky yellow; residue yellow on evaporation
Chlorophyll solution in 80 per cent acetone	Pale green solution, green residue on evaporation	Beautiful ruby-red; on evaporation, yellowish-red
Annatto in concentrated solution	Very dark blood-red	Dirty redish-yellow
Pure annatto, 1:20 aqueous solution	Very dark orange red	Dull orange-red
Butter colour made from above	Bright red	do.
Lactochrome solution water	Pale greenish yellow	Bright greenish white

extracted by the simple method recommended by Dan, Kon, and Moore [1937].

It has been suggested by Morgen and MacLennan [1928], etc. that the blue colours obtained are due to vitamins. They may also possibly be due to sterols. Cholesterol has a blue fluorescence; and crystals of plant phytosterol when examined showed a light purple colour.

Finally, the results of the examination of *ghee* itself are given in Table VI and discussed.

The first suggestion is that the colour range is wide and erratic. However, it is seen that (1) no fresh *ghee* gives any tint of blue, (2) old and very white *ghee* gives a blue shade and (3) a yellow colour in *ghee* reveals itself as a yellow colour in fluorescent light. The observations on carotene come to mind. It seems as if carotene masks the true colour of the *ghee*, and that its removal might yield better information. Some of the above samples of *ghee* were therefore bleached with 10 per cent of animal charcoal for 20 seconds and filtered. Also a petrol-ether solution of carotene (extracted from dried carrots) was added drop by drop to these bleached samples till the colours matched the

TABLE V

Unsaponifiable matter from ghee *and oils*

Sample	Colour by day light	Colour by ultra-violet light
Sindhi cow *ghee*, rather old	Yellow oily globules	Bright bluish violet
Murrah buffalo *ghee*, three years old	Pale yellow viscous liquid	Blue with green tinge
Dacca *ghee*, June, 1942	Yellow slimy liquid	Bluish yellow
Porbunder buffalo *ghee*, February, 1942	Colourless oil	Bright purple
Fresh buffalo *ghee*	Yellow oily drops	Bright bluish purple
Ghee prepared from soured cream	White milky liquid	Bright blue
Groundnut hardened oil	Pale yellow oil	Bright bluish purple
Cocoanut hardened oil	do.	do.
Cotton seed hardened oil	Silky white crystals	Fairly bright blue
Cocoanut oil	Yellow oily liquid	Bluish violet

TABLE VI

Fluorescence of ghee

Sample	Colour by day light	Colour by ultra-violet light
1. Fresh buffalo *ghee*	Pale yellow solid and liquid on melting	Pale greenish white
2. Fresh buffalo *ghee* gone slightly rancid	Pale yellow solid, almost colourless on melting	Pale greenish white with tint of blue
3. Cow *ghee*, fresh	Bright yellow liquid	Bright milky yellow
4. Old *ghees*—		
(*a*) Hariana cow *ghee*, 2½ years	Golden yellow liquid	Light blue
(*b*) Ayrshire cow *ghee*, 2½ years	Bright yellow	Bright canary yellow
(*c*) Sindhi cow *ghee*, 2½ years	do.	do.
(*d*) Murrah buffalo *ghee*, 2½ years	Dirty yellow liquid	Bright greenish blue

originals in a Lovibond Tintometer. In every case the original fluorescent colours were restored. The results of bleaching are given below (Table VII).

TABLE VII

Bleaching of ghee

Sample	Colour by day light	Colour by ultra-violet light
1. Sample 1 in Table VI	Colourless liquid	Fairly bright green tint
2. Sample 3 in Table VI	Very pale yellow liquid	Pale greenish yellow
3. Ayrshire cow .	Pale yellow .	Pale bluish green
4. Porbunder, February, 1942	Quite colourless .	Pale blue with tint of purple
5. Dacca, January, 1943	Very pale yellow .	Pale blue with green tint
6. Dacca, June, 1942	do. . .	do.

They indicate that the age of a sample is revealed in a progressive blue colour, often masked by the yellow colour of carotene. For example, the very white Porbunder Sample 4 (Table VII) gave the same colour even after bleaching; and the green tints of samples 3, 5 and 6 may be explained as resulting from the blue as a consequence of ageing together with the slight residual yellow of carotene. The following results (Table VIII) in every case confirm the deductions stated above, and indicate that a fresh fat should give a greenish-white shade on bleaching.

TABLE VIII

Additional fluorescence colours

Sample	Colour by day light	Colour by ultra-violet light
Cow *ghee*, fresh .	Bright yellow	Bright yellow
Cow *ghee*, bleached	Quite colourless	Pale greenish white
Akola (Sample 18) cow *ghee*, $2\frac{1}{2}$ years	Fairly bright	Pale yellow
Akola (Sample 18) cow *ghee*, bleached	Pale yellow	Pale green with blue tint
Akola (Sample 125) cow *ghee*, $2\frac{1}{2}$ years	do.	Pale blue with faint green tint
Akola (Sample 125) cow *ghee*, bleached	Colourless	Pale blue
Akola (Sample 96) cow *ghee*, $2\frac{1}{2}$ years	Pale yellow	Pale blue with green tint
Akola (Sample 96) cow *ghee*, bleached	Colourless	Pale blue
Buffalo *ghee*, fresh	Faint yellow	Greenish with tint of yellow
Buffalo *ghee*, fresh bleached	Colourless	Greenish without tint of yellow
Lyallpur *ghee*, May, 1942	Pale yellow	Pale blue with tint of green
Lyallpur *ghee*, bleached	Very pale yellow	Pale blue
Lyallpur *ghee*, April, 1942	Pale yellow	Pale blue with tint of green
Lyallpur *ghee*, bleached	Very pale yellow	Pale blue
Kirkee *ghee*, November, 1941	Hardly perceptible yellow	Bright bluish purple
Kirkee *ghee*, December, 1941	Slightly darker yellow than above	do.
Gauhati, July, 1942	Pale yellow	Pale yellow tinged with green
Gauhati, July, 1942 bleached	Very faint yellow	Pale blue with touch of green
Cow *ghee*, cream process	Yellow	Bright yellow
Cow *ghee*, curd process	do.	Bright yellow, faint green tint

TABLE VIII

Additional fluorescence colours

The ageing of *ghee* results in various changes, and these appear to be indicated in a blue shade. To test this hypothesis, *ghees* were maintained at 90°C. in thin films exposed to the atmosphere for 48 hours. While this does not involve the same organic processes as normal rancidification [Banks, 1944] the results are interesting in that they show the marked blue colour produced in the *ghees*, which were all quite colourless after the teattment.

TABLE IX

Ageing of ghee

Sample	Colour by day light	Colour by ultra-violet light
Fresh buffalo *ghee*	Colourless	Beautiful bluish violet
Ghee from soured cream	do.	do.
Fresh cow *ghee* .	do.	do.

SUMMARY

1. The fluorescence of *ghee* and its adulterants in ultra-violet light has been studied.

2. The pronounced effect of carotene on the colour has been established. The sample should always,

therefore, be bleached with animal charcoal (10 per cent for 20 seconds) prior to a viewing of the colour.

3. Old and rancid *ghee* gives progressively blue shades: most adulterants of *ghee* also fluoresce blue. Therefore any suggestion of a blue tint in a bleached *ghee* indicates either bad quality or adulteration.

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STUDIES IN THE TRANSPORT OF MILK IN WARM CONDITION FOR MARKETING PURPOSES

I. KEEPING QUALITY OF FARM-PRODUCED AND PROCESSED MILK

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IT is now recognized that in hygienic supply of milk from rural areas to urban population refrigerated system of transport occupies a most prominent position. Unfortunately under present conditions, this involves high initial cost as well as high recurring expenditure. Since the chief cause of spoilage of milk is bacterial contamination, it is desirable to find out if other methods of treatment such as heating the milk to the pasteurizing temperature, which destroys a major portion of the organisms, coupled with its transportation in a hot condition would be successful in preventing the spoilage. The idea is not a new one as Bernstein [1893] claimed good results in the transport of hot milk at 70°C. between Hamburg and Berlin, a railway journey of 10 hours. On the other hand, Ayers and Johnson [1923] reviewed the position and came to the conclusion that milk could not be kept in a good condition for 24 hours at a temperature of 50°-60°C. because of the growth of thermophilic bacteria. No record of similar work carried out in India under controlled conditions is available, but it is reported that it is a common practice adopted by some Military Dairy Farms to issue hot milk (145°F.) to customers during summer months.* The milk handled in this way is probably consumed in a few hours' time. Another method, practised by the *halvais*, specially in northern India, is to keep the milk simmering for 7-8 hours and then dispose it of as demanded by the customers. In this method, there is no doubt that considerable chemical changes are brought about.

As the 'holding' method of pasteurization, in which milk is heated to 145°F. and held at that temperature for half an hour, is considered to be suitable for destroying most of the organisms, it is probable that if this heated milk is kept at more or less the same temperature, the stability of the milk will be prolonged for a sufficiently long period, so that it can be transported over long distances without giving rise to undesirable chemical changes. If this is so, much of the expenditure on refrigeration could be avoided and the cost of transport is likely to be reduced. In order to obtain some definite information on the subject, systematic experiments have been started in these laboratories and in the present communication, some results on the keeping quality of farm-produced milk at elevated temperature as compared to milk heated to 150°F. and retained at that temperature for half an hour, cooled and then stored at low (48°-50°F.) temperature, are given.

EXPERIMENTAL

Experiments were carried out on a laboratory scale and then some trials were made on a large scale. The experimental work was divided as follows:

(i) Storage of processed milk at high (150°F.) and low (48°-50°F.) temperatures for eight hours in glass milk bottles.

(ii) Storage of processed milk at high (150°F.) and low (48°-50°F.) temperatures for 16 hours in small aluminium cans.

* *Report on the marketing of milk*, 1943

(iii) Storage of processed milk at room temperature (80°-90°F.) and low temperature (48°-50°F.) for 20 hours in small aluminium cans.

(iv) Storage of processed milk in bulk at a fluctuating storage temperature (130°-150°F.) for eight hours in large tinned steel milk cans.

Plan of work

Required quantities of raw, mixed milk as detailed under different sections were collected and heated to 150°F. and retained at that temperature for 30 minutes in a specially constructed milk pasteurizing can, provided with a stirrer, a tight-fitting lid and an arrangement for inserting the thermometer to record the temperature of the milk without opening the lid. This can was inserted in a bigger vessel containing water which was directly put over a charcoal fire. The milk while being heated was stirred at five minutes intervals to attain uniform temperature. After the process of heating and retaining the milk at the temperature was completed, it was immediately distributed in containers, which were previously sterilized. After filling the containers, they were closed with tight-fitting lids. Those samples which were to be maintained at an elevated temperature were at once transferred to a hot chamber maintained at 150°F. and the samples were immediately cooled down to between 48°-50°F. by immersing the containers in cold brine solution and then transferred to cold storage (48°-50°F.).

Section I

Eight pounds of milk were taken, heated, retained at the temperature and then distributed in glass milk bottles as detailed above. These were stored at 150°F. and 48°-50°F. for the total period of eight hours.

Section II

In this section the experimental treatment to the milk was the same as described above, the modifications being (i) the use of 12 lb. of milk (ii) the use of aluminium milk cans of 5 lb. capacity as containers and (iii) the storing of milk for a total period of 16 hours.

Section III

To study the behaviour on processed milk, held at room temperature (80°-90°F.) versus that held in cold storage (48°-50°F.), this part of the work was done. The modification made was the holding of one lot of milk, after heating and retaining at 150°F. for half an hour, at room temperature while holding the other lot of samples, after cooling, at cold storage temperature (48°-50°F.).

Section IV

These trials were made to study the effect of high storage temperature, within a certain fluctuating limit, which may be obtainable under field conditions on a large quantity of milk.

Sixty pounds of milk were employed for the trials. The method of heating followed here was slightly different. The whole milk can was immersed in hot water and the milk heated to 150°F. and held at that temperature for half an hour. After completion the can was removed and transferred to an insulated chamber which was heated by a charcoal fire. The inside temperature was not constant but fluctuated between 130° and 150°F.

In every case the following chemical, bacteriological and physical tests were applied to milk samples, drawn at various stages:

Chemical	Bacteriological	Physical (only applied to milk after hot storage)
1. Fat per cent	1. Total No. of organisms per c.c. of milk	1. Smell
2. Acidity (lactic acid per cent)	2. Presence of *B. coli* in milk. 3. Methylene blue reduction test. 4. Fermentation test	2. Taste.

The milk samples were drawn and examined at the following stages:

(i) Raw milk (before treatment).

(ii) Milk heated to 150°F. and held at that temperature for 30 minutes.

(iii) Milk after complete storage period at both the storage temperatures, i.e. 150°F. and 50°F.

(iv) Milk samples drawn at three and six hourly intervals in the case of trials under section III.

Results and discussion

Results of the chemical analysis of various samples are given in Table I, while the data on bacteriological examination are given in Tables II and III.

From the chemical results it will be observed that the fat percentage in milk was not altered by the two different treatments, namely storage at

TABLE I

Acidity and fat percentage in milk at different stages

Sample number	Raw milk		Milk heated to 150°F. for 30 minutes		Milk heated to 150°F. for 30 minutes, immediately cooled to 48°-50°F. and stored at 48°-50°F. for the period of						Milk heated to 150°F. for 30 minutes and: Kept at 150°F. for the period of				Kept between 130°-150°F. for the period of				Kept at room temperature (80°-90°F.) for the period of			
					8 hours		16 hours		20 hours		8 hours		16 hours		4 hours		8 hours		3 hours		6 hours	
	Fat percentage	Acidity percentage of lactic acid	Fat percentage	Acidity percentage of lactic acid	Fat percentage	Acidity percentage of lactic acid	Fat percentage	Acidity percentage of lactic acid	Fat percentage	Acidity percentage of lactic acid	Fat percentage	Acidity percentage of lactic acid	Fat percentage	Acidity percentage of lactic acid	Fat percentage	Acidity percentage of lactic acid	Fat percentage	Acidity percentage of lactic acid	Fat percentage	Acidity percentage of lactic acid	Fat percentage	Acidity percentage of lactic acid
1	6·20	0·192	6·20	0·178	6·20	0·170	—	—	—	—	6·25	0·170	—	—	—	—	—	—	—	—	—	—
2	4·95	0·188	5·00	0·178	4·95	0·173	—	—	—	—	4·75	0·178	—	—	—	—	—	—	—	—	—	—
3	6·00	0·142	6·00	0·142	6·00	0·152	—	—	—	—	6·40	0·152	—	—	—	—	—	—	—	—	—	—
4	5·00	0·152	5·30	0·162	5·30	0·142	—	—	—	—	5·30	0·152	—	—	—	—	—	—	—	—	—	—
5	6·90	0·165	6·90	0·164	6·90	0·164	—	—	—	—	6·80	0·165	—	—	—	—	—	—	—	—	—	—
6	3·80	0·156	3·80	0·152	3·80	0·156	—	—	—	—	3·70	0·152	—	—	—	—	—	—	—	—	—	—
Average	5·48	0·166	5·53	0·163	5·53	0·160	—	—	—	—	5·53	0·162	—	—	—	—	—	—	—	—	—	—
1	5·40	0·155	6·40	0·145	—	—	5·40	0·139	—	—	—	—	5·40	0·140	—	—	—	—	—	—	—	—
2	5·55	0·134	5·50	0·129	—	—	5·50	0·129	—	—	—	—	5·55	0·129	—	—	—	—	—	—	—	—
3	6·40	0·145	6·35	0·145	—	—	6·40	0·140	—	—	—	—	6·50	0·140	—	—	—	—	—	—	—	—
4	5·75	0·145	5·75	0·146	—	—	5·70	0·142	—	—	—	—	5·70	0·143	—	—	—	—	—	—	—	—
5	6·00	0·122	6·00	0·122	—	—	5·90	0·114	—	—	—	—	5·90	0·114	—	—	—	—	—	—	—	—
6	6·05	0·118	6·05	0·118	—	—	6·00	0·118	—	—	—	—	6·00	0·118	—	—	—	—	—	—	—	—
Average	5·86	0·137	5·84	0·134	—	—	5·82	0·130	—	—	—	—	5·84	0·131	—	—	—	—	—	—	—	—
1	5·40	0·112	5·45	0·108	—	—	—	—	5·45	0·112	—	—	—	—	—	—	—	—	5·45	0·112	5·45	0·128
2	4·75	0·115	4·70	0·112	—	—	—	—	4·75	0·110	—	—	—	—	—	—	—	—	4·75	0·120	4·75	0·125
3	4·80	0·095	4·85	0·100	—	—	—	—	4·85	0·090	—	—	—	—	—	—	—	—	4·85	0·105	4·85	0·115
4	4·60	0·130	4·60	0·125	—	—	—	—	4·75	0·120	—	—	—	—	—	—	—	—	4·70	0·135	4·70	0·145
5	5·75	0·085	5·75	0·090	—	—	—	—	5·75	0·090	—	—	—	—	—	—	—	—	5·70	0·095	5·85	0·095
6	5·00	0·092	5·00	0·085	—	—	—	—	5·00	0·095	—	—	—	—	—	—	—	—	5·00	0·095	5·00	0·105
Average	5·05	0·105	5·06	0·103	—	—	—	—	5·09	0·103	—	—	—	—	—	—	—	—	5·08	0·110	5·10	0·119
1	5·60	0·105	5·60	0·105	—	—	—	—	—	—	—	—	—	—	5·60	0·110	5·60	0·115	—	—	—	—
2	5·55	0·120	5·60	0·120	—	—	—	—	—	—	—	—	—	—	5·60	0·130	5·60	0·130	—	—	—	—
3	4·35	0·145	4·35	0·145	—	—	—	—	—	—	—	—	—	—	4·25	0·140	4·25	0·135	—	—	—	—
4	5·35	0·135	5·40	0·130	—	—	—	—	—	—	—	—	—	—	5·35	0·135	5·40	0·130	—	—	—	—
5	4·90	0·115	4·90	0·115	—	—	—	—	—	—	—	—	—	—	4·95	0·130	4·95	0·135	—	—	—	—
6	4·70	0·115	4·75	0·115	—	—	—	—	—	—	—	—	—	—	4·70	0·150	4·75	0·150	—	—	—	—
Average	5·08	0·123	5·10	0·122	—	—	—	—	—	—	—	—	—	—	5·03	0·133	5·09	0·133	—	—	—	—

Table II

Bacteriological data on milk at various stages

Sample number	Raw milk	Milk heated to 150°F. for 30 minutes			Milk heated to 150°F. for 30 minutes, immediately cooled to 50°F. and stored at 48°-50°F. for the period of						Milk heated to 150°F. for 30 minutes and then: Kept at 150°F. for the period of				Kept between 130°-150°F. for the period of				Kept at room temperature (80°-90°F.) for the period of			
					8 hours		16 hours		20 hours		8 hours		16 hours		4 hours		8 hours		3 hours		6 hours	
	Bact/ml.*	M. B. R.† time in minutes	Bact/ml.	M. B. R. time in minutes	Bact/ml.	M. B. R. time in minutes	Bact/ml.	M. B. R. time in minutes	Bact/ml.	M. B. R. time in minutes	Bact/ml.	M. B. R. time in minutes	Bact/ml.	M. B. R. time in minutes	Bact/ml.	M. B. R. time in minutes	Bact/ml.	M. B. R. time in minutes	Bact/ml.	M. B. R. time in minutes	Bact/ml.	M. B. R. time in minutes
1	3,270,000	45	340	485	350	330	—	—	—	—	210	330	—	—	—	—	—	—	—	—	—	—
2	670,000	165	1,720	405	1,610	330	—	—	—	—	410	330	—	—	—	—	—	—	—	—	—	—
3	460,000	90	50	390	190	300	—	—	—	—	110	300	—	—	—	—	—	—	—	—	—	—
4	750,000	165	690	375	290	300	—	—	—	—	240	300	—	—	—	—	—	—	—	—	—	—
5	2,630,000	135	570	340	420	270	—	—	—	—	720	270	—	—	—	—	—	—	—	—	—	—
6	1,820,000	115	8,100	285	7,000	260	—	—	—	—	8,200	—	—	—	—	—	—	—	—	—	—	—
Average	1,600,000	119	1,912	380	1,790	298	—	—	—	—	1,660	306	—	—	—	—	—	—	—	—	—	—
1	160,000	—	3,600	—	—	—	5,700	240	—	—	—	—	3,950	335		—	—	—	—	—	—	—
2	302,000	—	10,300	—	—	—	13,000	260	—	—	—	—	1,700	335		—	—	—	—	—	—	—
3	87,000	—	4,700	—	—	—	5,700	302	—	—	—	—	5,600	306	—	—	—	—	—	—	—	—
4	146,000	—	6,600	—	—	—	3,500	215	—	—	—	—	2,100	313	—	—	—	—	—	—	—	—
5	440,000	—	21,000	—	—	—	3,700	300	—	—	—	—	2,750	355	—	—	—	—	—	—	—	—
6	140,000	—	4,600	—	—	—	5,300	205	—	—	—	—	4,400	280	—	—	—	—	—	—	—	—
Average	212,500	—	8,470	—	—	—	6,150	254	—	—	—	—	3,400	321	—	—	—	—	—	—	—	—
1	3,800,000	126	21,900	355	—	—	—	—	23,000	215	—	—	—	—	—	—	—	—	2,300	220	52,000	70
2	2,900,000	163	37,000	320	—	—	—	—	4,000	250	—	—	—	—	—	—	—	—	31,000	220	460,000	120
3	280,000	175	1,800	340	—	—	—	—	18,000	220	—	—	—	—	—	—	—	—	30,000	284	310,000	115
4	860,000	120	2,600	335	—	—	—	—	15,000	189	—	—	—	—	—	—	—	—	8,000	246	100,000	150
5	530,000	133	3,200	385	—	—	—	—	16,000	208	—	—	—	—	—	—	—	—	4,400	240	49,000	125
6	900,000	105	3,300	250	—	—	—	—	2,200	200	—	—	—	—	—	—	—	—	16,000	175	280,000	95
Average	1,562,000	137	11,630	331	—	—	—	—	13,200	214	—	—	—	—	—	—	—	—	18,700	231	209,000	169
1	300,000	140	1,100	420	—	—	—	—	—	—	—	—	—	—	1,700	320	1,200	280	—	—	—	—
2	60,000	220	1,500	480	—	—	—	—	—	—	—	—	—	—	2,400	390	3,800	380	—	—	—	—
3	400,000	190	10,800	450	—	—	—	—	—	—	—	—	—	—	16,000	365	7,500	355	—	—	—	—
4	960,000	180	7,100	450	—	—	—	—	—	—	—	—	—	—	5,200	370	7,000	360	—	—	—	—
5	520,000	130	36,000	420	—	—	—	—	—	—	—	—	—	—	12,800	340	—	335	—	—	—	—
6	410,000	180	4,900	450	—	—	—	—	—	—	—	—	—	—	1,600	375	6,700	360	—	—	—	—
Average	442,000	173	10,200	445	—	—	—	—	—	—	—	—	—	—	6,600	360	5,240	343	—	—	—	—

* Bact/ml. indicates the number of bacteria per ml. of milk at 37°C. on standard milk agar.
†M. B. R. stands for methylene blue reduction.

Table III

Results of fermentation tests

Sample number	Raw milk		Milk heated to 150°F. for 30 minutes	Milk heated to 150°F. for 30 minutes, immediately cooled to 48°-50°F. and stored at 48°-50°F. for the period of			Milk heated to 150°F. for 30 minutes and then: Kept at 150°F. for the period of		Kept between 130°-150°F. for the period of		Kept at room temperature (80°-90°F.) for the period of	
	*B. coli**	Fermentation	Fermentation	8 hours	16 hours	20 hours	8 hours	16 hours	4 hours	8 hours	3 hours	6 hours
1	1 : 100	SG.SW.SC†	NG.NW.SC	NG.NW.SC	—	—	NG.NW.SC	—	—	—	—	—
2	1 : 100	G.SW.SC	SG.SW.SC	NG.NW.SC	—	—	NG.NW.SC	—	—	—	—	—
3	1 : 10	NG.NW.SC	NG.NW.SC	NG.NW.SC	—	—	NG.NW.SC	—	—	—	—	—
4	1 : 100	SG.SW.SC	NG.NW.SC	NG.NW.SC	—	—	NG.NW.SC	—	—	—	—	—
5	1 : 100	G. W.SC	NG.NW.SC	SG.SW.SC	—	—	NG.NW.SC	—	—	—	—	—
6	1 : 100	SG.SW.SC	NG.NW.SC	NG.SW.SC	—	—	NG.SW.SC	—	—	—	—	—
1	1 : 1000	SG.SW.SC	NG.NW.SC	—	NG.SW.SC	—	—	NG.SW.SC	—	—	—	—
2	1 : 10	SG.SW.SC	NG.SW.SC	—	NG.SW.SC	—	—	SG.SW.SC	—	—	—	—
3	1 : 100	SG.SW.SC	NG.SW.SC	—	NG.SW.SC	—	—	NG.SW.SC	—	—	—	—
4	1 : 1	NG.W.SC	SG.SW.SC	—	NG.SW.SC	—	—	NG.SW.SC	—	—	—	—
5	1 : 100	SG.SW.SC	NG. W.SC	—	NG.SW.SC	—	—	NG.SW.SC	—	—	—	—
6	1 : 10	SG.SW.SC	NG.SW.SC	—	NG.SW.SC	—	—	SG.SW.SC	—	—	—	—
1	1 : 1000	SG.SW.SC	NG.SW.SC	—	—	NG.W.SC	—	—	—	—	NG.SW.SC	NG.SW.SC
2	1 : 1000	SG.SW.SC	NG.SW.SC	—	—	NG.W.SC	—	—	—	—	NG.SW.SC	NG.SW.SC
3	1 : 10	SG.SW.SC	NG.SW.SC	—	—	NG.SW.SC	—	—	—	—	NG.SW.SC	NG.SW.SC
4	1 : 1000	SG.SW.SC	NG.SW.SC	—	—	NG.SW.SC	—	—	—	—	NG.SW.SC	NG.SW.SC
5	1 : 1000	SG.SW.SC	NG.SW.SC	—	—	NG.SW.SC	—	—	—	—	NG.SW.SC	NG.SW.SC
6	1 : 1000	SG.SW.SC	NG.SW.SC	—	—	SG.SW.SC	—	—	—	—	NG.SW.SC	NG.NW.SC
1	1 : 1000	VSG.VSW.SC	NG.VSW.SC	—	—	—	—	—	NG.NW.SC	NG.SW.SC	—	—
2	1 : 10	VSG.VSW.SC	NG.VSW.SC	—	—	—	—	—	NG.NW.SC	NG.NW.SC	—	—
3	1 : 1000	VSG.VSW.SC	NG.VSW.SC	—	—	—	—	—	NG.NW.SC	NG.NW.SC	—	—
4	1 : 1000	SG.SW.SC	NG.VSW.SC	—	—	—	—	—	NG.NW.SC	NG.NW.SC	—	—
5	1 : 1000	VSG.VSW.SC	NG.VSW.SC	—	—	—	—	—	NG.NW.SC	NG.NW.SC	—	—
6	1 : 1000	VSG.VSW.SC	NG.VSW.SC	—	—	—	—	—	NG.NW.SC	NG.NW.SC	—	—

* This is used to denote presence of *B. coli* in the dilution mentioned in the columns. Further results of the presence of *B. coli* are not given since they were found absent even in 1 ml. of milk after heating and after the storage periods.

†SG., Slight gas; G., Gas; VSG., Very slight gas;
+SW., Slight separation of whey; NG., No gas; VSW., Very slight separation of whey;
+SC., Solid curd meaning no gas holes and shrinking; NW., No separation of whey; W., Separation of whey.

high temperature (150°F.) and at low temperature (48°-50°F.). With regard to acidity there was a slight decrease on heating which may be due to the expulsion of CO_2 during the process. The acidity then remained fairly constant in the case of milk stored at high temperature, while in the case of milk in cold storage, there was a very slight decrease. Naturally the milk stored at room temperature showed a decided increase in acidity after three hours and still greater increase was noticed after six hours. In fact this milk actually curdled when stored for 20 hours, thus proving that processed milk cannot be stored at ordinary room temperature for more than about six hours. But samples of milk stored at 150°F. and 48°-50°F. for 16 hours showed more or less the same acidity, proving that hot storage at 150°F. is just as efficient as cold storage from this point of view, but it was noticed in the field scale trials that, even if 150°F. storage temperature was not rigidly maintained but allowed to fluctuate between 150°F. and 130°F., there was very little increase in acidity within eight hours of storage.

The bacteriological data show that the total number of organisms in raw milk decreased considerably on heating. The samples stored at 48°-50°F. at the temperature and retaining the milk for 8, 16, and 20 hours showed that there was neither increase nor decrease in the total count as compared to the total count in milk after heating. The total count of milk stored at 150°F. for 8 and 16 hours showed a decrease when compared to the bacterial count obtained after heating and retaining the milk at the temperature as referred to previously. The same decrease is noticeable in field scale trials. This proves that hot storage from the point of view of growth of surviving organisms after processing the milk, brought about not only inhibition of growth of the organisms but it did actually destroy them to a certain extent, while the cold storage only inhibited their further multiplication. This appears to show that either the farm milk, which is produced under hygienic condition, did not contain many thermophilic organisms, or even if they were present, they would not affect the storage quality of heated milk at a temperature of 150°F. for at least 16 hours. Storage of the processed milk at the ordinary temperature kept it in good condition only for about four hours.

As regards *B. coli*, raw milk which showed the presence of *B. coli* in 1 : 100 and 1 : 1000 dilutions showed its absence in even 1 ml. of milk just after processing and it continued to remain absent even after storage periods, thus proving that milk once processed, if not recontaminated, would show complete absence of *B. coli*.

The methylene blue reduction time shown by raw milk was considerably increased from about two hours to six hours after processing. In case of milks stored at 150°F. and 48°-50°F. for eight hours, the reduction time was more or less the same, while in case of 16 hours' storage, milk stored at 150°F. showed a longer reduction time than the milk stored at 48°-50°F. This observation is further supported by the fact that the total number of organisms in milk stored at 150°F. after 16 hours showed a greater reduction than the count in milk stored at 48°-50°F. Milk stored at atmospheric temperature curdled at the end of a '20 hours' storage period, while that stored at 48°-50°F. at the end of 20 hours showed a total reduction time of 3½ hours. Similar results for methylene blue reduction were noticeable both in case of storage of eight hours at 150°F. in laboratory scale as well as in case of field trials.

The fermentation test results showed that in the raw milk curd, there was presence of slight gas and a slight separation of whey. The curd formed after processing and storage periods did not show the above defects and the curd formed was more palatable.

A slight cooked odour was observed in all samples stored at 150°F. for different storage periods, while samples stored at 48°-50°F. retained their natural odour. There was practically no appreciable difference in the taste of milk.

The effects of storage at an elevated temperature on the mineral and vitamin contents of milk as well as the behaviour of village produced milk under these conditions, are being studied. If these results prove satisfactory and the nutritive quality of milk remains unimpaired, large scale field trials on the transportation of milk at an elevated temperature will be made.

Summary

(1) An investigation has been carried out on the keeping quality of farm-produced milk, processed at a temperature of 150°F. and held at about that temperature for prolonged periods.

(2) It has been observed that there is no change either in acidity of milk or fat percentage when the milk is kept in hot condition up to 16 hours.

(3) Bacteriological examination shows that there is a definite decrease in the number of organisms when the milk is kept at an elevated temperature as compared to that in cold storage.

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THE FATTY ACID COMPONENTS OF INDIAN BUFFALO *GHEES*

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The work consists of the analyses by ester-fractionation methods of Indian *ghee*.

In the choice of buffalo *ghee* for analysis, several factors were considered. Firstly this product is by far the most widely used type of *ghee* in India. Secondly the literature reveals only three former analyses of buffalo *ghee* [Bhattacharya and Hilditch, 1931; Heiduschka and Cicekdagi, 1940] of which two were Indian and even these were chosen at random and were not specially characteristic in any way. These three analyses have been quoted later in the course of the discussion.

In the choice of typical specimens the basis used was the content of lower fatty acids as reflected in the Reichert-Meissl and Polenske values. The sum of these two values for buffalo *ghee* varies from about 15 to about 40, and so it was thought desirable to study three specimens of normal (30·8), high (37·4) and low (20·7) R.M. respectively. This would not only be merely representative and indicative of any constancy of feature wherewith to detect adulteration with foreign fats, but interesting from the theoretical point of view in that it would indicate which acid or acids, if any, compensated for this high or low content of lower acids.

Experiment

The sample of low R.M. *ghee* obtained from the Porbandar State had been stored for a long time and was rancid at the time of analysis, and hence another fresh sample was procured and analysed. The fresh samples have been recorded as 1, 2 and 3 and the rancid sample as 4. The *ghees* were dried with anhydrous sodium sulphate, filtered and stored in glass-stoppered brown bottles in an ice-box till required.

The analyses were carried out by the ester-fractionation method with an electrically heated and packed column using the method employed by Smith and Dastur [1938] of direct methanolysis and separation of the lower acids in some quantity before undertaking the Twitchell separation. The separation of the esters is illustrated by the detailed statement of results (Table I, A, B, C, D) obtained in the case of the normal R.M. *ghee* sample 2. Considerations of space do not permit of the full details of the other analyses.

Table I(A)

Lower esters

Fraction	B. pt. at 2 mm. from (°C.)	Temp. of middle (°C.)	Weight (gm.)	Percentage of total esters	M. Wt.	I. V.	M. Wt. of saturated esters (by calculation)
L0	..	..	7·905	5·06	..	..	..
L1	30	48	0·323	0·21	133·1	12·57	129·3
L2	41	69	0·420	0·27	145·1	11·49	154·9
L3	48	78	0·472	0·30	177·6	9·46	177·2
L4	60	99	0·792	0·51	191·0	7·97	191·4
L5	61	118	0·979	0·63	211·0	6·38	210·9
L6	78	139	1·188	0·76	213·5	4·80	213·5
L7	96	143	1·576	1·00	226·9	7·69	227·8
L8	106	147	2·048	1·32	238·5	7·19	238·4
L9	110	151	1·999	1·28	241·0	5·39	241·1
L10	112	156	1·901	1·22	247·9	6·59	248·4
L11	122	161	1·823	1·17	251·6	7·29	253·0
L12	129	165	2·387	1·53	251·6	7·58	252·5
L13	133	169	3·106	2·00	253·1	8·32	254·3
L14	138	175	2·687	1·73	255·6	9·02	254·5
L15	142	176	3·801	2·44	260·7	9·31	259·9
L16	148	178	2·704	1·74	261·5	11·61	260·7
L17	150	181	2·994	1·92	264·6	14·07	264·0
L18	151	181	2·420	1·56	273·9	18·86	275·5
L19	152	182	1·559	1·00	272·6	16·21	273·5
			43·084	27·65			

TABLE I(B)

Saturated esters

Fraction	B. pt. at 2 mm. from (°C.)	Temp. of middle (°C.)	Weight (gm.)	Percentage of total esters	M. Wt.	I. V.	M. Wt. of saturated esters (by calculation)
S1	79	156	0·653	0·42	244·8	4·62	242·4
S2	97	162	0·900	0·58	250·0	3·47	249·5
S3	116	166	1·328	0·85	256·8	2·85	255·2
S4	126	168	2·019	1·30	262·9	2·90	262·0
S5	134	171	3·527	2·27	266·1	3·60	265·0
S6	139	174	2·794	1·80	272·7	5·72	271·1
S7	139	176	3·664	2·35	271·9	8·25	269·5
S8	143	183	6·710	4·31	276·8	9·62	274·3
S9	147	196	7·716	4·96	274·7	10·84	271·6
S10	157	202	8·645	5·55	279·9	13·82	277·1
S11	163	205	9·235	5·93	282·9	16·01	279·9
S12	167	206	10·056	6·46	287·2	19·50	284·7
S13	169	207	6·715	4·31	290·8	16·84	289·6
S14	168—falling	210	3·539	2·27	294·9	22·52	294·4
S15	Residue	..	2·980	1·92	296·2	20·61	296·3
			70·481	45·28			

TABLE I(C)

Unsaturated esters

Fraction	B. pt. at 2 mm. from (°C.)	Temp. of middle (°C.)	Weight (gm.)	Percentage of total esters	M. Wt.	I. V.	Molecular weight of saturated esters (by calculation)		
U1	87	176	0·652	0·42	244·7	30·11		246·3	
U2	109	178	0·636	0·41	247·5	32·89		251·2	
U3	120	180	1·021	0·66	257·2	39·21		259·4	
U4	129	182	1·460	0·94	267·8	52·57		267·7	
							M. wt. Of fully saturated	H atoms required for full saturation	I. V. of C_{18} and C_{20} esters
U5	138	184	1·836	1·18	279·9	64·80	281·3	1·4	74·44
U6	146	187	1·946	1·26	285·3	67·70	286·8	1·5	73·96
U7	150	189	2·368	1·53	286·8	77·78	288·6	1·8	85·19
U8	153	192	4·100	2·63	283·8	76·37	285·5	1·7	86·66
U9	154	199	3·379	2·18	290·9	78·79	292·7	1·8	82·68
U10	157	203	1·927	1·24	290·9	80·71	292·8	1·9	84·55
U11	160	207	3·861	2·48	295·0	83·20	296·9	1·9	83·95
U12	161	208	4·318	2·77	295·4	85·09	297·4	2·0	85·51
U13	161	211	2·573	1·62	294·3	85·57	296·3	2·0	86·82
U14	166	216	2·997	1·93	294·1	87·10	296·1	2·0	88·80
U15	166—falling	218	2·818	1·79	298·4	87·50	300·5	2·1	..
U16	Residue	..	6·262	4·23	301·7	88·60	303·8	2·1	..
			42·154	27·07					

Total weight of esters = 43·084 + 70·481 + 42·154
= 155·719 or 155·7 gm.

TABLE I(D)

Resolution of fractions

Fraction	Percentage of total esters	Saturated esters								Unsaturated esters							
		C_4	C_6	C_8	C_{10}	C_{12}	C_{14}	C_{16}	C_{18}	as C_{20}	C_{10}	C_{12}	C_{14}	C_{16}	C_{18}'	C_{18}''	as C_{20}'
L0	5·06	5·06	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..
L1	0·21	..	0·19	..	..	..	..	..	..	..	0·02	..	..	..	..	..	..
L2	0·27	..	0·02	0·23	..	..	..	..	..	..	0·02	..	..	..	..	..	..
L3	0·30	..	..	0·08	0·20	..	..	..	..	..	0·02	..	..	..	..	..	..
L4	0·51	..	..	..	0·37	0·11	..	..	..	..	0·03	..	..	..	..	..	..
L5	0·63	..	..	..	0·06	0·54	..	..	..	..	..	0·03	..	..	..	..	..
L6	0·76	..	..	..	..	0·73	..	..	..	..	..	0·03	..	..	..	..	..
L7	1·00	..	..	..	..	0·45	0·49	..	..	..	..	0·06	..	..	..	..	..
L8	1·32	..	..	..	..	0·14	1·09	..	..	..	..	..	0·09	..	..	..	..
L9	1·28	..	..	..	..	0·04	1·17	..	..	..	..	..	0·07	..	..	..	..
L10	1·22	..	..	..	..	..	0·86	0·28	..	..	..	..	0·08	..	..	..	..
L11	1·17	..	..	..	..	..	0·68	0·46	..	..	..	..	0·08	..	..	..	..
L12	1·53	..	..	..	..	..	0·84	0·58	..	..	..	..	0·11	..	..	..	..
L13	2·00	..	..	..	..	..	0·99	0·86	..	..	..	..	0·15	..	..	..	..
L14	1·73	..	..	..	..	..	0·81	0·72	..	..	..	..	..	0·20	..	..	..
L15	2·44	..	..	..	..	..	0·74	1·46	..	..	..	..	..	0·24	..	..	..
L16	1·74	..	..	..	..	..	0·47	1·06	..	..	..	..	..	0·21	..	..	..
L17	1·92	..	..	..	..	..	0·32	1·31	..	..	..	..	..	0·29	..	..	..
L18	1·56	..	..	..	..	..	..	0·98	0·27	..	..	..	..	0·31	..	..	..
L19	1·00	..	..	..	..	..	..	0·72	0·11	..	..	..	..	0·17	..	..	..
S1	0·42	..	..	..	..	..	0·38	0·01	..	..	..	..	..	0·03	..	..	..
S2	0·58	..	..	..	..	..	0·40	0·16	..	..	..	..	..	0·02	..	..	..
S3	0·85	..	..	..	..	..	0·40	0.41	..	..	..	..	..	..	0·04	..	..
S4	1·30	..	..	..	..	..	0·84	0·92	..	..	..	..	..	..	0·04	..	..
S5	2·27	..	..	..	..	..	0·38	1·85	..	..	..	..	..	..	0·09	..	..
S6	1·80	..	..	..	..	..	..	1·61	0·07	..	..	..	..	..	0·12	..	..
S7	2·35	..	..	..	..	..	0·04	2·08	..	..	..	..	..	..	0·23	..	..
S8	4·31	..	..	..	..	..	..	3·17	0·62	..	..	..	..	..	0·52	..	..
S9	4·96	..	..	..	..	..	..	4·05	0·28	..	..	..	..	..	0·63	..	..
S10	5·55	..	..	..	..	..	..	8·41	1·28	..	..	..	..	..	0·86	..	..
S11	5·03	..	..	..	..	..	..	3·03	1·79	..	..	..	..	..	1·11	..	..
S12	6·46	..	..	..	..	..	..	2·26	2·73	..	..	..	..	..	1·47	..	..
S13	4·31	..	..	..	..	..	..	0·97	2·49	..	..	..	..	..	0·85	..	..
S14	2·27	..	..	..	..	..	..	0·19	1·48	..	..	..	..	..	0·60	..	..
S15	1·92	..	..	..	..	..	..	0·80	0·66	..	..	..	..	..	0·46	..	..
U1	0·42	..	..	..	..	..	0·25	0·06	..	..	..	..	0·11	..	..	..	..
U2	0·41	..	..	..	..	..	0·18	0·10	..	..	..	..	0·13	..	..	..	..
U3	0·66	..	..	..	..	..	0·15	0·24	..	..	..	..	0·13	0·14	..	..	..
U4	0·94	..	..	..	..	..	0·03	0·39	..	..	..	..	..	0·52	..	..	..
U5	1·18	..	..	..	..	..	0·21	..	0·10	..	..	..	..	0·21	0·66	..	..
U6	1·26	..	..	..	..	..	0·12	..	0·21	..	..	..	..	0·13	0·80	..	..
U7	1·53	..	..	..	..	..	0·15	..	0·01	..	..	..	..	0·15	1·22	..	..
U8	2·63	..	..	..	..	..	0·35	..	..	..	..	..	..	0·34	1·02	0·02	..
U9	2·18	..	..	..	..	..	0·12	..	0·07	..	..	..	..	0·12	1·87	..	..
U10	1·24	..	..	..	..	..	0·06	..	..	..	..	..	..	0·07	1·02	0·09	..
U11	2·48	..	..	..	..	..	0·03	..	..	..	..	..	..	0·02	2·43	..	..
U12	2·77	..	..	..	..	..	0·02	..	..	..	..	..	..	0·01	2·74	..	..
U13	1·62	..	..	..	..	..	0·02	..	..	..	..	..	..	0·03	1·55	0·02	..
U14	1·93	..	..	..	..	..	0·04	..	..	..	..	..	..	0·04	1·78	0·07	..
U15	1·79	..	..	..	..	..	..	..	..	..	..	..	..	..	1·58	0·05	0·16
U16	4·03	..	..	..	..	..	..	..	..	..	..	..	..	..	2·92	0·21	0·90
Percentage by wt.	100·00	5·06	0·21	0·31	0·63	2·01	11·93	34·24	12·17	..	0·09	0·12	0·95	3·25	27·51	0·46	1·06
Molar percentage as acids.	100·00	13·50	0·43	0·52	0·86	2·37	12·32	31·49	10·08	..	0·12	0·14	0·99	3·01	22·97	0·39	0·81

Results

The following tables show the characteristics and structures (expressed as molar percentages of acids) of the four samples of *ghee* analysed.

Table II (A)

Characteristics of the samples of ghee *analysed*

—	1	2	3	4
1. Origin	Agricultural College, Kirkee	Dinshaw Dairy, Bangalore	Satvirda Hills, Porbandar State	Satvirda Hills, Porbandar State
2. Type of feeding, etc.	Grass and concentrates, a little groundnut cake, pasture	Grass and concentrates, varied oil-seeds in small amounts	Heavy cottonseed feeding, little or no pasture	Heavy cottonseed feeding, little or no pasture
3. General characteristics	Excellent flavour and texture, bright yellow colour, fresh	Excellent flavour and texture, bright yellow colour, fresh	Good flavour, small grains tending to be hard	Rancid, choking flavour. Very small grains, very hard to the feel, quite white in colour
4. Noteworthy feature	High R.M.	Normal R.M.	Low R.M.	Low R.M. but rancid
5. R.M.	37·4	30·8	20·7	22·7
6. P.V.	1·9	1·2	0·6	0·8
7. I. V.	27·4	28·9	37·0	34·9
8. S. V.	227·3	223·7	212·6	216·7
9. Free fatty acidity as percentage of lactic acid	0·07	0·07	0·09	1·7

Table II (B)

Fatty acid composition of the ghees *analysed (as molar percentages)*

Acid	1	2	3	4
Butyric . .	15·4	13·5	11·5	10·1
Caproic . .	1·1	0·4	..	0·7
Caprylic . .	1·4	0·5	0·1	2·2
Capric . .	1·4	0·9	0·5	1·8
Lauric . .	1·9	2·4	0·8	2·6
Myristic . .	9·2	12·3	4·8	7·1
Palmitic . .	31·9	31·5	25·1	22·5
Stearic . .	12·5	10·1	19·0	16·8
as Arachidic .	0·1	..	1·1	1·0
Decenoic . .	0·1	0·1 0·03	0·1	0·3
Dodecenoic .	0·1	0·1 0·05		0·2
Tetradecenoic .	0·6	1·0	0·5	0·8
Palmitoleic .	3·0	3·0	2·9	5·1
Oleic . .	16·8	23·0	32·0	28·6
Linoleic . .	1·2	0·4	1·0	0·2
as Gadoleic .	3·3	0·8	0·6	..

Discussion

It has previously been indicated by several workers [Achaya, Katrak and Banerjee, 1943; Smith and Dastur, 1938] that there exists in general a marked inverse relation between the iodine value of a sample of butterfat and its R.M. value. While samples 1 and 3 analysed are in excellent agreement with the average figures for these constants, sample 2 has rather a low iodine value in comparison with its R.M.

Also, sample 4 of butterfat was, as has been stated, rancid at the time of analysis and hence the high content of lower saturated and unsaturated acids cannot be considered as normal, and represents products of breakdown, probably oleic acid (which is known to be liberated in hydrolytic rancidity), which the high acidity of 1·7 per cent (as lactic acid) indicated to have occurred to a marked extent.

These analyses reveal for the first time the presence in buffalo milk-fat of unsaturated acids lower than oleic. Their presence in cow milk-fat [Hilditch and Longenecker, 1938] and goat milk-fat [Riemenschneider and Ellis, 1936] has been adequately proved, and the bond of unsaturation established at the 9-10 position from the carboxyl group as in oleic acid. While the quantity of material available in the present series did not permit the establishment of absolute proof of the occurrence of the structurally identical acids, the trend of iodine values in the lower ester fractions (coupled with the proved efficiency of the fractionation), which exhibited a series of maxima and minima, and the probability of similarity of features in all ruminant mammals,

were considered sufficient warrant for the assumption. The molar percentages for decenoic, dodecenoic, tetradecenoic and hexadecenoic acids came out as about 0·1, 0·1, 0·7 and 3·0 respectively, which agree fairly well with the values 0·3, 0·3, 1·0 and 3·0 of Smith and Dastur [1938] for cow milk-fats of generally greater unsaturation. The percentages now indicated must, however, be regarded only in the nature of an approximation of the true proportions of these acids in buffalo milk-fat though not far from them.

A further interesting feature of these analyses is the presence of an acid higher than linoleic, calculated here as a C_{20} acid with one double bond (gadoleic), in the absence of material for more detailed analysis. The other three analyses (loc. cit.) in the literature recorded the highest unsaturated acids as linoleic and the molar percentages as 2·2 and 0·2.

The most striking feature of the present results is the gradually rising contents of oleic acid as the amounts of the lower acids decrease, a relationship reflected in a more general way in the R.M. and iodine values. This is the more marked in that samples 1 and 2, of about the same iodine value, could be expected to contain the same amounts of oleic acid; actually, the proportions are quite different and the low iodine value of sample 2 is accounted for by its low linoleic, rather than oleic, content.

Smith and Dastur [1938] observed very similar results with the cow in their work on inanition. They indicated there that several theories of the origin of these lower acids could account for the features observed among which was the theory of Hilditch and his colleagues [1941] that the lower acids are formed by the breakdown of preformed oleo-glycerides in the mammary gland. The general nature of the present results strongly favours this view, since it postulates a direct relationship between the two components in question. Moreover, the presence of lower unsaturated acids probably identical with those of cow and goat milk-fat is satisfactorily accounted for in that they represent 'fragments of transformed oleo-glycerides which have escaped complete saturation to lower saturated acids'. The recent tentative postulate of Hilditch and Meara [1944] that the precursor in blood might well be a linoleo-glyceride does not affect the results since the production of an oleo-glyceride from a linoleo-glyceride represents a comparatively simple hydrogenation.

While the first two *ghees* analysed are of a pattern, samples 3 and 4 fall into another class by reason of their high percentages of stearic acid. That there appear to be two such fairly distinct types of buffalo milk-fats is supported by the three other analyses extant, which are quoted below (Table III) after conversion in all cases to molar percentages.

TABLE III

Fatty acid composition of ghee (*as molar percentages*)

—	5	6	7
Butyric	10·8	10·9	11·9
Caproic	3·3	2·8	2·6
Caprylic	0·5	1·5	0·7
Capric	1·3	2·4	Trace
Lauric	2·4	3·3	3·7
Myristic	7·7	10·5	7·9
Palmitic	19·0	28·7	25·0
Stearic	20·9	9·3	14·2
Arachidic	2·1	0·7	2·6
Oleic*	29·7	27·7	31·2
Linoleic	2·3	2·2	0·2

These analyses show that the high percentage of stearic acid need not necessarily occur where the content of lower acids is small or that of oleic large as has happened in samples 3 and 4 of the present series. In other words the proportions of stearic acid appear to be fixed by a mechanism independent of that operating in the fixing of the proportions of oleic acid on the one hand and the lower acids on the other.

It is necessary to explain the emphasis on the high stearic rather than low myristic contents of the samples under discussion. Hilditch and co-workers (loc. cit.) have shown that the tendency in the butterfats and depot fats of herbivorous mammals is for the palmitic acid content to remain constant at about 25 per cent; in addition, Patel, Patel and Dave [1944] have recently shown that the buffalo is much more susceptible to feeding vagaries than the cow and reflects slight changes in its feed by fairly marked changes in its milk-fat. It seems probable, therefore, that the low palmitic content while still tending to constancy is a mathematical consequence of the high proportion of stearic acid, coupled in this case with a high oleic content besides.

The mechanism of this high production of stearic acid is of interest. It can be shown that it cannot directly be traced to any specific fatty-acid or glyceride structure of an oil, since similar oils have widely different effects on milk-fat. Since the precursor of both milk-fat and body fat is the neutral triglyceride fraction of blood [Maynard et al, 1938; Aylward, Blackwood and Smith, 1937; Peterson, Palmer and Eckles, 1929; etc.], it would be safe to draw comparisons where similar effects on both are produced by an ingested fat. Soybean oil does not produce any untoward effect on cow milk-fat in

*The oleic percentages are about 4 units too high since lower unsaturated acids were not accounted for.

the stearic acid content [Hilditch and Thompson, 1936]; yet it has a fatty-acid and glyceride structure almost identical with cotton seed oil. Nor can the matter be explained by direct infiltration of the acid from cottonseed oil, since the latter has a molar content of stearic acid of only 2 per cent.

Whatever may be the causes of differences of mobilization of different oils, the source of stearic acid in this case is almost certainly dietary oleic or linoleic acid. Since the effects are produced both in depot fats and in milk-fats, it may seem attractive to consider the hydrogenation as having occurred before passage of glycerides into the blood. But in view of the simultaneously lowered production of lower acids [Brown and Deck, 1930; Brown, 1931] in milk-fats, it seems more probable that the hydrogenation process takes place during selective withdrawal of glycerides in the depot tissues on the one hand and in the mammary gland on the other.

There is another aspect of the question: part of the excessive fat in the diet probably passes into the liver as fatty acids, and these exert their modifying influence on fat being synthesized from carbohydrate material.

Two other features of interest in the analytical figures are (1) the high proportions of arachidic acid in the three fats conforming to the high-stearic type; (2) the high gadoleic acid content of *ghee* 1. This may be an infiltration from the groundnut cake that partly comprised the feed of the animal but too much stress cannot be laid on the slender evidence drawn from a single ester fraction.

The practical implications of the three present analyses taken in the light of the detection of adulteration are suggestive in the following way. The two features with any claim to constancy are the C_{18} unsaturated acid content and the $C_{14}+C_{16}+C_{18}$ saturated acid percentages. The difficulties in the way of estimation of the former are immense; the latter feature is also of little value since the $C_{16}+C_{18}$ contents of many oils and hydrogenated oils are in the neighbhourhood of the 53 per cent for the sum just mentioned. The only other figure of any interest is the linoleic acid content, a determination of which is possible by the iodine and thiocyanogen values together. This is not because the percentage of this acid is by any chance constant, but because of the high linoleic content of common oils which is well known; fortunately the only exception (coconut oil having a linoleic acid content of about 1·5 per cent) can easily be detected by the R.M.-P.V. relationship. An addition of as little of 10 per cent of other oils should greatly raise the small linoleic content of samples of *ghee*. Finally, emphasis can still be laid on the empirical analytical 'constants' of butterfat, particularly the R.M.-P.V.-I.V. relationship.

SUMMARY

1. The analyses of four samples of Indian buffalo *ghee* of high, normal and low R.M. value (two samples) are given in full.

2. The presence of the lower unsaturated acids, decenoic, dodecenoic, tetradecenoic and hexadecenoic, has been tentatively postulated and their rough proportions indicated.

3. The presence of an acid higher than linoleic has been indicated and calculated as gadoleic acid.

4. A striking inverse relationship between the lower acids and oleic acid, similar to the results obtained on inanition by Smith and Dastur, is discussed.

5. The effect of ingesting cottonseed oil has been shown to be a greatly increased stearic acid content. The mechanism of this high production has been discussed.

6. The determination of the linoleic acid content has been theoretically postulated as likely to help in the determination of the genuineness or otherwise of a sample of buffalo butterfat.

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DISTRIBUTION AND SEASONAL INCIDENCE OF SURRA IN INDIA

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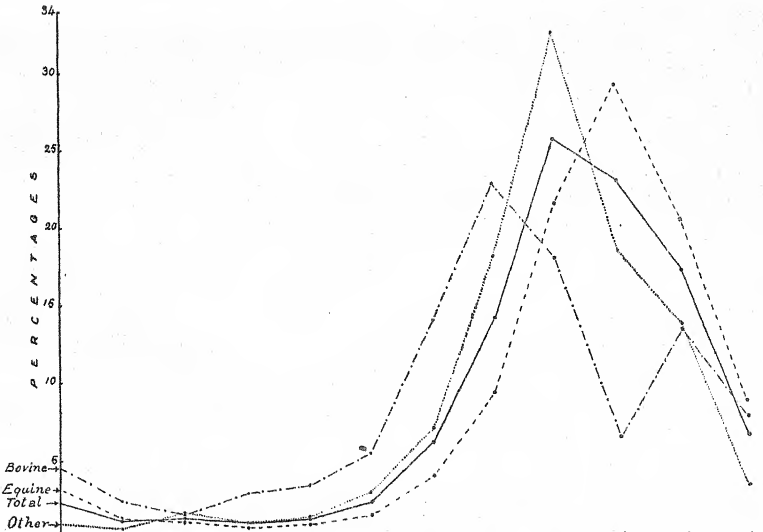
(With three text-figures)

The data, which form the subject of this note, were obtained from directors of veterinary services of provinces and states and pertain to a period of three years from January 1940 to December 1942. The figures are shown in Table I.

A map (Fig. 3) has been prepared from these figures showing the intensity of surra in various parts of India. Of the Indian States, only Hyderabad, Baroda, Gwalior, Alwar, Travancore and Mysore furnished detailed information; Kashmir sent some figures, but no details were available; no information was available from the remaining Indian States or from Ajmer. It will be seen from the map that many infected areas are isolated, in reality they may have been found to be continuous with the main areas if more detailed information had been available; that is, discontinuity of distribution in most cases is probably more apparent than real. The map also gives the impression that the disease is more prevalent in the areas where rainfall is scanty and the camel population is high. Unfortunately almost no information is available from Rajputana, a camel-rearing area with scanty rainfall. It will also be seen that the disease is prevalent throughout India.



	JAN.	FEB.	MAR.	APR.	MAY	JUNE	JULY	AUG.	SEP.	OCT.	NOV.	DEC.
Bovine	4·5	2·2	1·5	2·8	3·3	5·3	13·7	22·4	17·6	6·2	13·0	7·5
Equine	3·2	1·4	1·1	0·7	0·9	1·4	3·9	9·1	21·1	28·7	20·0	8·5
Other	1·1	0·7	1·7	0·7	1·3	2·9	6·9	17·8	32·2	18·1	13·4	3·2
Total	2·4	1·2	1·3	1·0	1·2	2·3	6·0	13·8	25·3	22·5	16·7	6·3

Fig. 1. Seasonal incidence of surra in India—bovine, equine, other and total—1940-1942

from a report by Christophers (1911)]. It will be seen that the rise and fall in the number of surra cases coincide exactly with that of malaria cases.

SUMMARY

1. From data so far available, the regional distribution of surra in India has been mapped. The Punjab shows a heavy incidence of the disease.

2. The occurrence of surra is seasonal. The peak period for bovine surra is in August, for equine surra it is October, and for others (camel, etc.) it is in September. In the aggregate, surra reaches its highest seasonal incidence in September.

3. The seasonal curve of surra and human malaria in the Punjab coincide.

ACKNOWLEDGEMENT

Thanks are due to the directors of veterinary services for help in providing incidence figures.

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INVESTIGATIONS ON FAMINE RATIONS

MANGO-SEED KERNEL

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ACCORDING to a recent estimate, the concentrates available in India are sufficient for only 29·1 per cent. of the adult bovine population. This does not take into account the requirements of growing animals, of equines and of the 47·9 million sheep and 37·7 million goats. Although, during post-war developments, it is considered that there will be an all-round increase in the yield of foodgrains and fodder, it will be some time before developmental plans can materialize. In view of the keen shortage of feeding-stuff for livestock the nature of which in times of famine has been described by Kehar [1944], it was considered desirable to investigate whether mango-seed kernel could be utilized for feeding animals during scarcity periods.

It may be pointed out that mango-seeds are at present thrown away as waste and, according to a rough estimate, the amount available may be about two million tons per annum, which would yield about one million tons of the kernel. Besides, the mango is popularly styled 'king of fruits' and its plantation is increasing every year. Mango-seeds collected from waste heaps were broken and the kernel chemically analysed (Table I).

TABLE I

Composition of mango-seed kernel

Crude protein	8·50
Ether extract	8·85
Fibre	2·81
Nitrogen-free extract	74·49
Total ash	5·35
Total	100·00
Calcium (Ca)	0·190
Phosphorus (P)	0·298

These values may be compared with those of some common grains (Table II).

TABLE II

Composition of some common grains

	Crude protein	Ether extract	Fibre	Nitrogen-free extract	Ash	Calcium (Ca)	Phosphorus (P)
Mango-seed kernel	8·50	8·85	2·81	74·49	5·35	0·19	0·30
Barley	9·48	1·67	5·23	79·09	4·53	0·18	0·37
Maize	11·11	4·39	1·90	80·66	1·94	0·01	0·41
Oats	10·07	6·55	12·71	65·88	4·79	0·11	0·41
Rice	8·33	0·88	0·38	89·13	1·28	0·16	0·21
Wheat	9·65	1·27	2·41	84·70	1·97	0·23	0·41
Corn*	8·60	3·60	2·00	63·00	1·30	0·01	0·25
Rye*	8·30	1·30	0·60	77·30	0·90	0·02	0·29

*American figures

The kernels were lightly crushed and placed before country bullocks which after two to three weeks acquired a taste for them. During the exploratory period of about 15 days it was observed that animals weighing about 700-800 lb. could not eat more than 6-7 lb. (3-3·5 lb., dry matter). This amount, however, did not provide the total amount of protein necessary for maintenance. It was, therefore, proposed to replace about 50 per cent of oil-cake by mango-seed kernels.

Three country bullocks were then selected for a long-term feeding experiment, the rations shown in Table III being given daily.

TABLE III

Rations given to selected country bullocks

Animal No.	Body weight	Rape-cake	Mango-seed kernel	Wheat *bhusa ad lib.* (approximately)
1	776 lb.	1 lb.	6 lb.	6 lb.
2	568 lb.	¾ lb.	6 lb.	6 lb.
5	832 lb.	1 lb.	6 lb.	6 lb.

In addition to the above ration, each animal was given 1 oz. of salt daily.

RESULTS AND DISCUSSION

Observations on bullocks. The ration was fed for about three months. It was observed that adult animals, which ordinarily maintained weight on the Institute schedule ration, gained an average of 33 lb. in weight on the experimental ration (Table IV).

The animals also put on fine condition and had a healthy appearance. After about five weeks' feeding on this ration, a metabolism experiment was conducted on the animals by the usual procedure to find out the nutritive value of mango-seed kernel. The results showing the digestibility coefficient, nitrogen, calcium and phosphorus balance of the whole ration, and the digestibility coefficient of mango-seed kernel are given in Tables V, VI and VII, respectively.

TABLE IV

Gain in weight of bullocks kept on experimental ration

Date	Weight in pounds			
	Animal No. 1	Animal No. 2	Animal No. 5	
*1-8-44	776	568	832	
8-8-44	770	560	800	
15-8-44	736	550	780	
22-8-44	762	556	832	
29-8-44	784	586	838	
5-9-44	776	584	832	
11-9-44	784	564	820	Metabolism period
21-9-44	784	566	824	
28-9-44	788	566	832	
5-10-44	792	572	836	
12-10-44	792	580	836	
19-10-44	816	582	846	
26-10-44	822	584	870	
Gain in weight	46	16	38	

* Date of first feeding

It will be observed from Table VII that the mean percentage digestibility of crude protein, ether extract and nitrogen-free extract are 72·1, 56·3 and 70·6, respectively. Replacement of oil-cake by mango-seed kernel to the extent of 50 per cent. gives a wide positive nitrogen balance and suggests that further economy can be effected by reducing the quantity of oil-cake. The digestible nutrients per 100 lb. of dry matter as compared with those other common grains is given in Table VIII.

From the above figures it appears that the digestible protein obtained from mango-seed kernel is only slightly poorer than that in oats and barley, while in total digestible nutrients and starch equivalent the kernel compares satisfactorily with corn and oats.

TABLE V

Digestibility coefficient of whole ration

Animal No.	Dry matter	Organic matter	Crude protein	Ether extract	Fibre	Nitrogen free extract	Total carbohydrates
1	52·9	55·4	56·2	59·2	39·6	60·6	55·0
2	58·4	61·3	56·6	63·9	50·7	65·9	61·7
5	57·1	57·5	62·1	65·5	41·9	61·6	56·3
Mean	56·1	58·1	58·3	62·9	44·1	62·7	57·7

TABLE VI

Nitrogen, calcium and phosphorus balance

	Animal		
	No. 1	No. 2	No. 5
Nitrogen intake—			
Mango-seed kernel	22·26	15·97	20·03
Rape cake	23·65	17·74	23·65
Wheat straw	14·10	10·81	12·93
TOTAL	60·01	44·52	56·61
Nitrogen excretion—			
Faeces	26·29	19·32	21·48
Urine	10·10	14·50	17·10
TOTAL	36·39	33·82	38·58
Nitrogen balance	+23·62	+10·70	+18·03
Biological value of the mixed protein.	94·0	74·8	80·6
Calcium intake—			
Mango-seed kernel	3·11	2·23	2·80
Rape cake	3·09	2·32	3·09
Wheat straw	4·43	3·40	4·06
TOTAL	10·63	7·95	9·95
Calcium excretion—			
Faeces	7·70	5·58	7·14
Urine	2·00	1·63	2·50
TOTAL	9·70	7·21	9·64
Calcium balance	+0·93	+0·74	+0·31
Phosphorus intake—			
Mango-seed kernel	4·88	3·50	4·39
Rape cake	4·72	3·58	4·72
Wheat straw	1·93	1·48	1·77
TOTAL	11·53	8·56	10·88
Phosphorus excretion—			
Faeces	10·15	7·44	9·10
Urine	0·06	0·04	0·05
TOTAL	10·21	7·48	9·15
Phosphorus balance	+1·32	+1·08	+1·73

TABLE VII

*Digestibility coefficient of mango-seed kernel**

Animal No.	Crude protein	Ether extract	Nitrogen-free extract
1	66·6	51·3	66·4
2	68·9	58·1	77·0
5	80·9	59·4	68·3
Mean	72·1	56·3	70·6

*Since the percentage of fibre is only 2·8, it has not been considered.

TABLE VIII

Digestible nutrients of mango-seed kernel as compared with those of other grains and seeds

	Digestible protein	Starch equivalent	Total digestible nutrients
Barley	7·4	84·6	86·0
Maize	8·2	93·3	94·3
Oats	7·8	73·4	78·5
Corn*	6·6	..	74·2
Rye*	7·1	..	87·0
Oats*	7·0	..	72·2
Mango-seed kernel	6·1	67·5	70·0

*American figures

These observations put mango-seed kernel in the category of the important concentrates for livestock and make available about 70 million lb. of digestible protein and 760 million lb. of starch equivalent per year from a hitherto unutilized source of food. It has been calculated that the digestible protein obtained from 80 lb. of oats is equal to that obtained from 100 lb. of mango-seed kernel and the starch equivalent from 86 lb. Moreover, the keeping quality of mango-seed kernels seems to be satisfactory, as no deterioration has taken place after ten months' storage.

Observations on rats

To investigate the possibility of human consumption of mango-seed flour, experiments are in progress on rats. Preliminary observations over a period of 15 weeks show no difference between the rate of growth of rats on the stock diet as compared with those on a diet in which two-thirds of the maize has been replaced by mango-seed kernel.

SUMMARY

Investigations have been made to find if mango-seed kernel, hitherto rejected as waste, could be utilized as a feed for livestock.

Mango-seed kernel has been fed to bullocks with advantage to the extent of 50 per cent. of the total digestible protein. The animals developed a liking for the kernels after a couple of weeks' feeding. During about 12 weeks' feeding adult animals gained, on an average, 33 lb. in body weight and developed a fine condition.

Mango-seed kernel is a rich source of protein and carbohydrate. According to available figures, it is estimated that 70 million lb. of digestible protein and 760 million lb. of starch equivalent will be available per annum from this hitherto unrecognized

source. The digestible protein obtained from 80 lb. of oats is equal to that obtained from 100 lb. and starch equivalent from 86 lb. of mango-seed kernel.

The high digestibility coefficient of the protein and nitrogen-free extract and the biological value of the protein give the seed a place in the list of important food stuffs.

Work on the feeding of mango-seed kernel to rats is in progress.

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A NOTE ON A NEW METHOD OF TESTING WOOL FOR MEDULLATION

By P. N. Nanda, M.R.C.V.S., Gurbax Singh, L.V.P. and S. B. Mogre, M. Sc., Government Livestock Farm, Hissar

(Received for publication on 21 October 1944)

(With Plate XIV)

Of the recognized methods of determining the percentage medullation in wool, only McMahon's Medullometer can be of use if a large number of samples from individual animals are to be dealt with. The other methods are too lengthy and require several days to analyse a single fleece. The use of the medullometer too is restricted to laboratories where electric current and technically trained staff are available. With a view to guide field workers, the authors have devised a simple rapid test to estimate approximately the medullation in wool. It is based on Elphick's benzol method. It is known that a clean sample of wool when placed under benzol exhibits an intensity of whiteness in proportion to the amount of medullation in the sample. Marked difference in whiteness can easily be judged with the naked eye. For selecting sheep carrying good quality wool, the easiest course is to take small samples from these sheep, wash the samples in petrol, take an equal quantity by weight of all of them and place them side by side, under benzol, in a black enamelled tray. From the intensity of whiteness in the samples, one can at once pick out the samples from sheep carrying better wool. This method can be used to pick out better quality sheep, but for recording the percentage of medullation in individual animals, which is so essential for breeding on scientific lines, it does not serve the purpose. To overcome this, certain standards have been devised. These standards show samples under benzol with different amounts of medullation, varying from zero to 100 per cent. For studying the approximate percentage of medullation in a sample, the same weight of the sample, as in the standards, is placed under benzol and its percentage determined by comparison. The method, therefore, involves two main operations :

(*a*) Making of standard samples from different breeds of sheep and

(*b*) Studying of samples in the field in comparison with the standard samples.

Making of standard samples. Two hundred small shoulder samples are taken from a particular breed of sheep. After getting rid of the free vegetable matter, each sample is first washed with a 1 per cent. neutral soap solution at 50°C. and then rinsed twice in petrol at the same temperature. After drying them at the room temperature, 100 milligrams of each sample are taken for making the standards. All these samples are put under benzol in an enamelled tray which can accommodate about 20 to 30 at a time. Judging from the intensity of whiteness shown, some samples showing varying amounts of hairiness, can be picked out. These selected samples are then arranged in series according to percentage of the medullation in them. The first one represents zero per cent and the last one 100 per cent. The intervening samples are then allotted arbitrary percentage medullation in an ascending order to indicate increased hairiness. The percentages fixed for the samples are according to the intensity of whiteness exhibited by them. To obtain sharp contrasts, the first sample should consist of pure wool and the last of pure hair. The estimation of medullation would be more accurate if a large number of samples are taken to make up the standards.

For more reliable results the percentage medullation in the series may be fixed by actually ascertaining the exact amount of medullation by the microprojection method. These standards can then be used for the estimation of medullation in any given sample of wool by comparison. To have standards handy in the field, photographs of prepared sets can be used with equal efficiency.

The technique consists in first taking a small sample from the sheep as close to the skin as possible. After cleaning it to get rid of all free vegetable matter and dirt, it is given a free rinsing in petrol for one to two minutes. It is then allowed to dry. One hundred milligrams of the sample are then weighed out by means of the portable balance. This quantity is spread out a little between the

thumbs and fingers and all the fibres are brought parallel to one another. The spreading out is done to the same extent as in the standard print (Plate XIV). This resultant sample must present an homogeneous appearance. It is placed in an enamelled tray and covered by glass to press down the fibres. Benzol is then poured in the tray, till the sample is immersed. By comparing the intensity of whiteness of the sample with the standards, the medullation is estimated. By using a suitable size of tray (8 in.×10 in.×1 in.), 10 to 15 samples can be examined at a time. A single hand can easily test 100 to 150 samples in a day. Average medullation in a single fleece can be worked out by testing eight to ten representative samples taken from different parts of the body of a sheep. To begin with, testing of the shoulder sample is enough for classing and culling sheep under village conditions.

SUMMARY

With a view to guide field workers a simple rapid test to estimate approximately the medullation in wool has been devised.

The test is based on the fact that a clean sample of wool, when placed under benzol exhibits an intensity of whiteness in proportion to the amount of medullation in the sample.

AVIAN LEUCOSIS COMPLEX (FOWLD-PARALYSIS) IN INDIA

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(Received for publication on 17 September 1945)

(With Plates XV-XVII)

The veterinary literature in this country contains records of paralysis in fowls attributed to a number of different causes, such as nutritional deficiencies [Mohey-Deen, 1933 ; Bachan Singh, 1940 ; Mahajan, 1934], neurolymphomatosis [Krishniengar, 1937 ; Naidu, 1938], and Ranikhet disease [Kaura and Iyer, 1937]. While reviewing these cases, Kaura [1941] remarked that 'unfortunately very little trouble has been taken to differentiate and classify the various types of paralysis and that the simultaneous occurrence of true fowld-paralysis in the same bird where some other diagnosis has been given has been overlooked'. A systematic survey was, therefore, undertaken to investigate the incidence and distribution of this disease in the country.

Survey

The first case diagnosed was a hen received in the laboratory for post-mortem examination in April 1941. Since then, the disease in its diverse forms has been diagnosed in Bombay, the Punjab, Hyderabad (Dn), the United Provinces, Central Provinces, Cochin, Mysore and Madras. Both sexes and all the common breeds as well as their crosses were seen to be affected. The survey was, however, confined to the organized farms only and little information is available about the incidence in village flocks.

Field observations

The prevalence of the disease was first investigated on a small commercial farm, which was started in 1936 with about 100 birds, and has been subjected to considerable in-breeding. In 1942, there were about 60 birds, mostly of White Leghorn and Rhode Island Red breeds. The fertility, hatchability and viability records were unsatisfactory. Mortality was high, deaths occurring at irregular intervals. Typical cases of leucosis were diagnosed which were later confirmed as (lymphoid) leucosis on histological examination. The affected fowls went light and ultimately lost control of their limbs. Lameness started as a jerky walk, followed by inability to use one or both limbs, with the characteristic clutching appearance of the claws. As the disease advanced, the fowls were unable to move and lay in a helpless condition, often with one leg stretched in front and the other behind (Plate XV, fig. 1). Drooping of one or both wings and twitching of the neck and head were also seen. Occasionally the eyes were affected, the pupil in such cases becoming distorted and the iris losing its pigment and power to expand or contract. The appetite and general appearance were unaltered even in the last stages of the disease. In some fowls diarrhœa was noticed in the later stages.

In another flock, where records of mortality from this condition were maintained over a period of three years (1941-44), the disease persisted year after year, prevailing at all seasons. The losses, though small, occurred at irregular intervals. Of 542 mature fowls, which died or were killed during the above period, leucosis was diagnosed *post-mortem* in 139 or 25·6 per cent.

In another area, one of us recognized the disease in widely-separated localities. Investigation revealed that the foundation stock at all these places was obtained from one central farm. Dead and living fowls obtained from the latter showed evidence of leucosis.

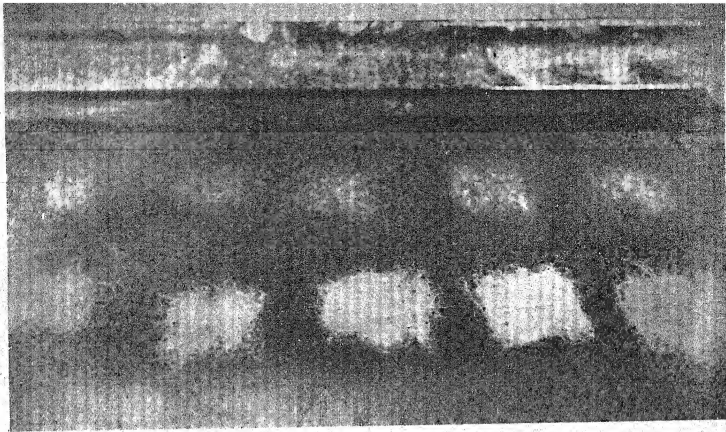

FIG. 1. Standard wool samples showing varying percentages of medullation as seen under benzol.

From left to right

1st row	5	15	30	40	50
2nd row	60	70	80	90	100

Note. The authors regret that owing to non-availability of suitable material a photograph could not be prepared to the desired standard.

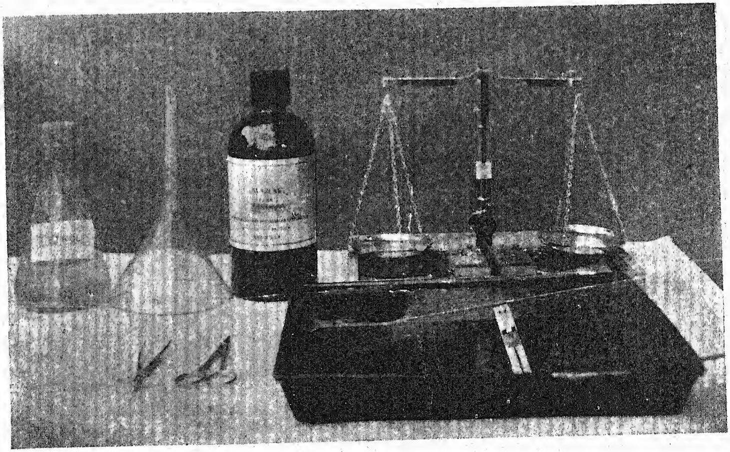

FIG. 2. Field outfit for the rapid test of medullation in wool.

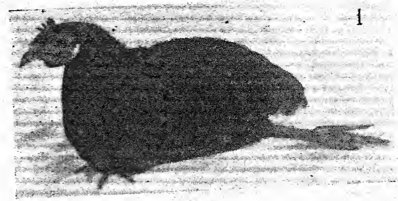

Fig. 1. Typical case of paralysis

Fig. 2. Enlargement of liver and spleen

Fig 3. Liver studded with tumours

Pathology

The following description is based on a study of material from 154 natural cases of the disease from all over the country.

Gross lesions. Frequently the most striking change noticed *post-mortem* was a uniform enlargement of the liver and spleen due to a soft, greyish-yellow infiltrating tissue (Plate XV, fig. 2). The liver was swollen to several times its normal size, weighing in some cases as much as 1¼ lb. (normal weight 2 oz.). The borders were rounded and there were small grey areas on the surface and dipping into the organ producing a greyish roughened hob-nailed appearance.

The liver was friable, resulting occasionally in rupture and internal haemorrhage. In many such cases, the kidneys (Plate XVI, fig. 5) were also affected. The bone-marrow was constantly involved, being swollen, pale-grey or brick-red in colour and abnormally solid. Anaemic changes were indicated by pallor of the comb, skin and visible mucous membranes.

The commonest manifestation, however, was the occurrence of tumour-like masses in the internal organs. In order of frequency in which the viscera were affected was liver, ovary, mesentery, spleen, pancreas, kidney, intestine, gizzard, proventriculus, voluntary muscle and skin. The tumours were well embedded, white or pink-grey, friable and varied in size from that of a pea to that of a hen's egg (Plate XV, fig. 3 and Plate XVI, fig. 4). Often the affection was localized, but sometimes the involvement of adjacent organs rendered it difficult to demarcate the boundaries of different structures.

In the nervous form of the disease, characterized by paralytic symptoms during life, changes were seen mostly in the sciatic, vagus, brachial and lumbo-sacral nerves. Instead of having a healthy glistening white colour, they were greyish yellow, oedematous and enlarged to several times their original thickness (Plate XVI, fig. 6). The normal transverse striations were often indistinct. Growths, millet-seed in size, were occasionally seen on the surface of such nerves. The posterior root ganglia of the cord were found asymetrically enlarged in a few cases.

In the ocular form shown by blindness during life, the iris was thickened, oedematous and depigmented, the pupil being distorted.

Thickening of the long bones, particularly the shanks, was seen in two localities in fowls which also showed other forms of the diease (Plate XVI, fig. 7).

Histology. The enlargement of the liver was usually due to massive infiltration of the stroma with immature blood cells which predominated in the peripheral blood and abounded in the bone marrow, liver, spleen, kidneys, etc. In the liver, which is the commonest site of infiltration, both intravascular and perivascular accumulations were seen. The visceral tumours were usually composed of closely packed small and large basic-staining non-granular mono-nuclear cells (lymphocytes and lymphoblasts). The invading cells infiltrated the parenchyma, and in some cases ultimately replaced it. There were no degenerative changes in the normal elements and these remained intact until entirely replaced by the infiltrating cells. In the liver the distribution of these cells varied from mild periportal accumulations to massive infiltrations, completely masking the normal tissue (Plate XVII, figs. 8 and 9). The lesions in the nerves consisted essentially of similar cellular infiltrations among the nerve fibres (Plate XVII, figs. 10 and 11). The brain, however, was unaffected even in the nervous form.

In the eyes, the iris showed lymphoidal cell infiltrations; the cornea was normal in structure, the retina usually unaltered and the optic nerve showed only slight cellular infiltration.

Discussion

The consensus of expert opinion [Bullis, 1944] groups the several manifestations of this disease under the term 'avian leucosis complex'. As the disease is characterized by definite tissue changes and as its transmission is diffcult or uncertain [Stubbs, 1938], histopathological evidence alone is considered sufficient for diagnosis. In this country, the clinical syndrome, gross lesions and histological features of the prevailing disease were seen to be typical of avian leucosis complex and comparable strictly with the disease existing in Great Britain. All the different manifestations were encountered, but a striking feature was the preponderance of the visceral form of the disease compared with the neural and ocular forms which are more commonly met with in other countries. The reasons for this are not known.

All the common breeds were seen to be affected, but, as the survey was confined to organized poultry farms maintaining chiefly fowls of exotic breeds, information on the incidence in indigenous breeds is scanty. This is an urgent question for investigation and warrants an extension of the survey to village flocks..

It was observed in the course of this survey that the advent of the disease may be hardly noticed in a flock and the danger of its presence rarely appreciated, as only a small percentage of birds seemed to be affected at a time. The appearance of other epidemic diseases, such as Ranikhet disease, is a spectacular event, calling for urgent measures, whereas in this disease accurate mortality records alone reveal the true measure of its import. In one of the flocks the disease has been held to be responsible for 25·6 per cent of losses in mature fowls during a period of three years. In some flocks it may be far worse, as the following statement from the *Report U. S. Department of Agriculture* [Winton, 1943]

shows, 'Lymphomatosis, one form of 'avian leucosis complex' manifested by paralysis, grey eyes leading to blindness, and enlarged livers, continues to be the cause of close to 40·0 per cent. of the total loss. Thus the yearly loss from this disease complex alone amounts to more than 52 million dollars'. For the institution of prompt control measures the importance of detecting the disease in a flock at an early stage is evident.

Summary

(1) The existence of 'avian leucosis complex' (fowld-paralysis) in widely-separated localities in India has been established on histopathological grounds.

(2) All the common breeds were noticed to be affected, but the survey was confined to organized poultry farms primarily maintaining exotic breeds. The incidence of the disease in *desi* fowls in rural flocks remains to be investigated.

(3) In one flock the disease persisted year after year and prevailed in all seasons. It was responsible for 25·6 per cent. of losses in adult birds during a period of three years.

(4) In at least one area the disease seemed to have been spread through the distribution of stock from a central farm.

(5) The clinical syndrome, gross lesions and histology of the disease prevailing in this country are described. These are typical of 'avian leucosis complex' and strictly comparable with those of the disease seen in Great Britain. All the different manifestations of the disease (visceral, neural, ocular and bone forms) were seen, histological examination revealing evidence of cellular infiltrations (lymphoid, myeloid and erythroid).

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NEW RECORDS OF NEMATODE PARASITES FROM INDIAN RUMINANT

By M. M. Sarwar, L.V.P., Imperial Veterinary Research Institute, Izatnagar

(Received for publication on 20 July 1944)

Marshallagia marshalli (Ransom, 1907) Orloff, 1933

This species has been recorded from several countries, and Monning [1940] has given a comprehensive account of its morphology. According to him, its distribution in S. Africa is restricted to desert areas only. It was obtained by the writer from the abomasum of the hill goat (*Capra sibirica*) at Mukteswar, Kumaun.

Haemonchus longistipes (Railliet and Henry 1909)

This parasite has already been recorded from camels and sheep. The writer obtained it from the abomasum of goats, sheep and cattle at Lahore, Peshawar and Karachi. Some of the measurements considered useful in specific diagnosis are given in Table I.

H. similis Travassos, 1914

This species has previously been recorded from hill bulls in this country [Bhalerao, 1933]. It has now been obtained by the writer from cattle and buffaloes at Sialkot (Punjab). In the writer's specimens, the spicules are from 0·27 to 0·28 mm. long, and the gubernaculum is about 0·115 mm. long. The barbs of the right and the left spicule are 0·05—0·55 mm. and 0·04—0·042 mm. long respectively.

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Table I

Measurements useful in specific diagnosis

	Host			
	Camel	Cattle	Sheep	Goat
Length of spicules	0·6—0·65	0·61—0·625	0·59—0·725	0·5—0·57
Barb of right spicule	0·09—0·1	0·08—0·1	0·085—0·1	0·075—0·1
Barb of left spicule	0·038—0·04	0·020—0·055	0·039—0·04	0·031—0·041
Length of stem of dorsal ray	0·168—0·213	0·152—0·175	0·145—0·215	0·14—0·21
Length of the main branches of dorsal ray	0·066—0·096	0·062—0·075	0·07—0·15	0·065—0·085

All the measurements are given in mm.

FIG. 4. Ovary with tumours

FIG. 5. Enlargement of kidneys

FIG. 6. Enlargement of right sciatic nerve

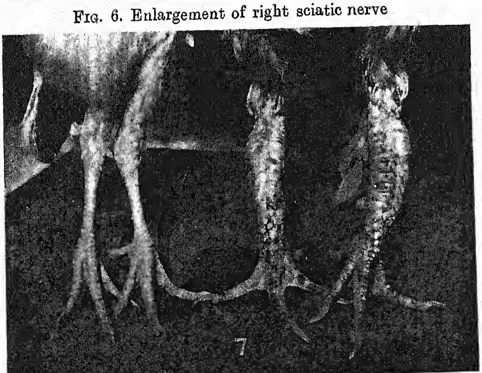

FIG. 7. (Left) Normal shanks; (Right) Enlargement of shanks bones

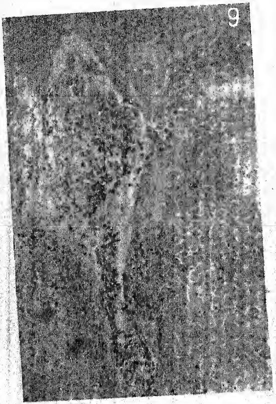

FIG. 9. Section of liver showing cellular infiltrations of portal tracts

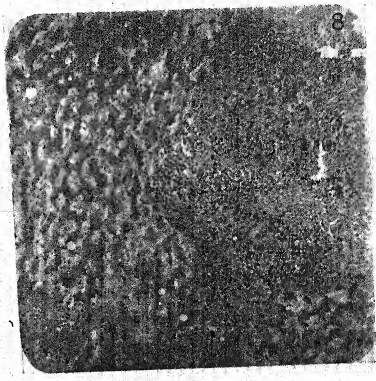

FIG. 8. Section of liver showing cellular infiltration in parenchyma

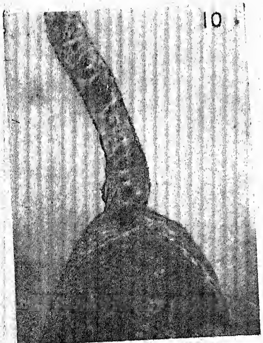

FIG. 10. Section of enlarged sciatic nerve

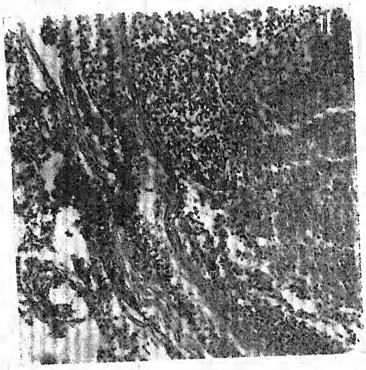

FIG. 11. Higher magnification of Fig. 10 showing cell. infiltration

DEMODECTIC MANGE OF GOATS IN INDIA

By M. K. Sreenivasan, G.M.V.C., and S. W. H. Rizvi, G.B.V.C., Imperial Veterinary Research Institute, Mukteswar

(Received for publication on 8 September 1945)

(With Plate XVIII)

Demodectic mange of goats has been known in Europe since 1881 when Niederhausern [1881] recorded the first case at Berne. Nocard and Railliet [1885] recorded the second case at Alfort, and Railliet [1895] described the mite and named it *Demodex folliculorum* var. caprae. Thereafter different workers reported the occurrence of this infection in other parts of Europe. In U.S.A. the first case was recorded by Cram [1925], and recently Durant [1944] has recorded some more. To our knowledge demodectic mange of goats has not so far been reported in India.

History

A batch of 50 female goats was purchased in March 1944 from a goat market in Bareilly district of the United Provinces. All these goats were picked out from different herds of far off localities arriving at the market on the same day. They were brought to Mukteswar and housed in an out-kraal, where there was no previous history of such infection. The number of goats purchased from any one herd did not exceed five. All the animals are of non-descript plains breed with short-haired coats, and are being maintained by the Institute for breeding. In July 1944, some of the kids of this herd were found having mangy eruptions on and under the ears, but scrapings of the affected parts were negative for mites. The non-specific nature of the lesions in kids prompted us to examine all the goats thoroughly with the result that two goats were found with a squamous type of itch on the surface and border of the ears, while one goat showed nodules on the face and neck. The material from both types of lesions was found to contain *Demodex*. One of us (S.W.H.R.) has an opportunity to examine about 500 goats at the same market recently and was able to detect a few suffering from demodectic mange. This diagnosis was confirmed microscopically. The infection appears to exist in Farukhabad and Etah districts of United Provinces from where these goats had come.

Clinical features

Three types of lesion were encountered, viz. (1) discrete, nodules, (2) squamous dermatitis and (3) acute dermatitis over coalesced nodules with slight matting of the hair.

The goat showing the nodular form was rather weak and had two kids to suckle. The nodules varied in size from a millet seed to that of a pea and were quite common and discrete on the face, neck and shoulders (Plate XVIII, fig. 1); a few were noticed on other parts of the body. On pressing the nodules a thick, greyish, ribbon-like material oozed out. This material was a mass of mites at all developmental stages with nymphal casts. The contents of a pea-sized nodule might roughly contain 22,000 mites. Very little loss of hair was noticed in this form but there was occasional itching.

In the squamous type, loss of hair was well marked. The lesions varied in size from a silver two-anna bit to an eight-anna piece and even bigger in some cases. The lesions were more or less circular and covered with dry reddish-grey discharge which made them appear raised. Scraping the lesion was quite painful and revealed a vascular base. The mites were not numerous in lesions of this type and no nodules could be detected in the affected area. The lesions were confined mostly to the ears and in some cases to the back and face (Plate XVIII, fig. 2). Hardenbergh and Schlotthauer [1925] reported a case of the squamous type of *Demodex* infection in goats in U.S.A. In the acute form, nodules of small size coalesced together and burst releasing a discharge which caused matting of the hair. There was more itching in this form and the goats lost condition. Probably the hair follicles were not affected as there was no loss of hair. Sometimes the lesion also extended to the teats (Plate XVIII, fig. 3).

The course of the disease in its nodular form appears to be indefinite, but in the squamous and acute diffuse forms it is shorter and can perhaps be controlled. It is necessary to mention that, as soon as the disease was detected, all goats with the slightest suspicion of infection were removed from the healthy stock but on subsequent examinations of the 'healthy' lot it was possible to pick out a few more infected animals. In all six out of 45 goats were declared diseased. The youngest animal found naturally affected was one and half year of age. No kid, even though in contact with an infected mother, has so far been found to be affected and this is in conformity with the observations of Hardenbergh and Schlotthauer [1925].

Transmission

Experimental transmission of demodectic mange is said to be very difficult and no positive results appear to have been reported. Mohler [1940] mentions that attempts to transfer the *Demodex* of cattle by

bringing infected nodules into direct contact with healthy skin, as well as by all other possible methods, failed. We have succeeded, however, in transmitting the goat *Demodex* to four out of six healthy goats in the following ways.

Group 1. Three young goats (two females and one male) and one hill-bull were used. Fresh infective material, obtained from a goat, was applied on both sides of the neck up to the bases of the ears after closely clipping the hair. A second application was similarly made a week later.

Group 2. Three young goats (two females and one male) and one hill-bull received the same material as Group 1, but the skin was shaved before applying the infective material. A second application was made a week later.

Hill-bulls were included in these experiments to see whether the mites of one species affected another, as supposed by Zurn, Babes, Lewandowsky and Scott [quoted by Hirst, 1921].

All the experimental animals were kept under strict isolation to avoid any chance infection. A month later none was showing signs of the disease. Later examinations were made at weekly intervals and at the end of four months one goat in Group 1 showed a few millet—sized nodules at the bases and pinnae of the ears and on one shoulder; *Demodex* was found on microscopical examination of the contents. One goat in Group 2 showed similar lesions at the end of five months and one more in each of Groups 1 and 2 developed the disease at the end of eight months. The two male goats, one in each group, and three kids born approximately five months after the experimental infection of the mothers, did not show infection. It is interesting to note that in none of the four goats experimentally infected with the disease, did lesions develop at the seat of application of the infective material. This may probably be due to the close clipping and shaving which made the part unsuitable for the mites to establish themselves and forced them to migrate to the neighbouring parts covered with hair.

Out of the two bulls used, one in Group 1 showed a few millet-sized nodules on shoulders and dewlap after four months. The material collected from this bull showed demodectic mites. It was at first thought that this was experimentally transmitted infection, but on careful microscopical examination the mites proved to be cattle *Demodex*, being morphologically identical with specimens collected from a calf at Hissar in 1936 by Mr M. Abdussalam who kindly lent us his collection for comparison. From this it may be concluded that this bull was incubating a natural infection of *Demodex* at the time of coming under experiment. But for this detailed study of the mites and their comparison with identified specimens, we might have erroneously believed like some other workers that *Demodex* of one host species can affect another.

In the case of the artificial infection in goats the lesions were small and of non-progressive type unlike the natural infection. In one animal they disappeared in about nine months. These goats were in good condition and are again in kid. The non-progressive nature of the disease may be due to the goats being in prime condition.

Discussion

In its principal characters the mite (Plate XVIII, fig. 4) agrees with the description given by Railliet [1895] and Cram [1925] and the lesions also agree in the main details with goat demodecosis reported in foreign countries.

The source of the original infection could not be traced but it is presumed that one or two of the goats were infected before purchase and that they passed the infection on to others by long cohabitation. Judging from the experimental data given above the period of incubation in natural cases appears to be not less than four months.

Mere occasional contact does not introduce the disease, as two Barbari bucks which came in close but occasional contact for one year with the infected goats have shown no sign of infection. Further a batch of experimental goats was already housed in the same kraal since September 1933, and came in occasional contact with the infected flock without getting infected. The non-infectivity of the kraal is shown by the fact that the experimental goats referred to above are still healthy. Both pregnant and non-pregnant goats were found affected; this is in contradicton to the belief of Rissling and Martin [quoted by Cram, 1925], who thought that only pregnant goats suffered.

Summary

1. Demodectic mange of goats is recorded for the first time in India.

2. The mode of spread of the infection appears to be through long cohabitation.

3. Sucklingkids in contact with diseased mothers have not been affected.

4. The infection has shown no special affinity for pregnant and long-haired goats as reported in foreign countries.

5. Experimental transmission was shown to be possible, infection occurring after an incubation period of 4-8 months.

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FIG. 1. Nodules on face and neck

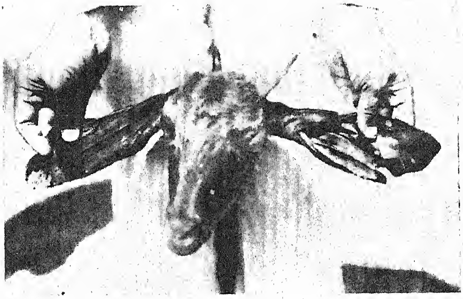

FIG. 2. Squamous lesion on ears

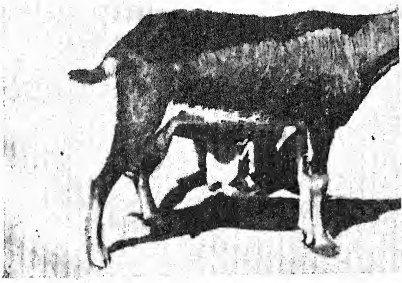

FIG. 3. Diffuse acute lesion

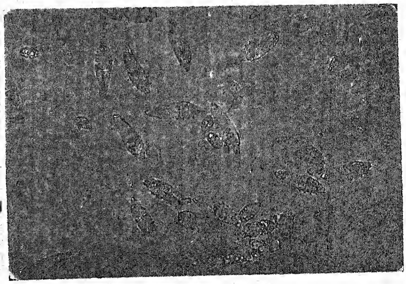

FIG. 4. Demodex caprae in all developmental stages (×57)

Niederhausern, V. (1881). Cited in Neumann (1905). *Treatise on the parasite and parasitis diseases of the domesticated animals*, Baillière, Tindall and Cox, London

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MENINGO-ENCEPHALITIS IN A BITCH

By G. K. Sharma, P.V.S., Punjab Veterinary College, Lahore

(Received for publication on 29 September 1945)

A bitch was admitted as in-patient on 12 November 1944.

History. She had whelped normally on the 10 November 1944 and suddenly went blind two days later.

Symptoms. When let loose she kept walking round in an anti-clockwise direction. The pupils were dilated and there was complete loss of vision without any evident abnormality (amaurosis). She had periodic fits of excitement accompanied by loud cries. There was staggering gait in the hind limbs and the head was occasionally pushed into the corner of the kennel. Marked spasms and stiffness of the neck muscles were also observed.

Later on the circle in which she walked became narrower and narrower till at last she used to rotate around her own axis. After walking a few steps the head was depressed and a complete sommersault was performed. Her body temperature ranged between 103° and 104°F. but in the last stages it came down to 102°F. Paralytic symptoms set in so that she was unable to stand. She died on 20 November 1944 under general paralysis.

Post-mortem. The meninges were found congested, adherent to the brain substance and studded with small haemorrhages. The uterus contained a small quantity of brownish discharge and the uterine mucosa was inflamed. It appears that the primary focus of infection was the uterus which gave rise to meningo-encephalitis.

The histopathological examination of the cerebral cortex showed acute congestion of the blood vessels with perivascular cellular infiltration. No other degenerative changes were seen.

A culture from the cerebral cortex was prepared on agar and blood agar media and subcultures were made on the same media which were sent to the Imperial Veterinary Research Institute, Mukteswar, for determinative bacteriology. The organisms were found to be gram-negative rods fermenting dextrose, lactose and mannite with the production of acid and gas.

It was therefore concluded that the organisms belonged to *B. coli* group.

Summary

A case of meningo-encephalitis in a bitch has been described.

It appeared from a post-mortem examination that the primary focus of infection was the uterus.

From a study of cultures from cerebral cortex for determinative bacteriology, it was concluded that the organism belonged to *B. coli* group.

Acknowledgement

My thanks are due to J. S. Garewal, Esqr., I.V.S., M.R.C.V.S., Principal, Punjab Veterinary College, Lahore, for providing necessary facilities at the College and to J. R. Haddow, Esqr., M.R.C.V.S., Officer-in-Charge, Bacteriology and Pathology Sections, Imperial Veterinary Research Institute, Mukteswar, for determinative bacteriological work.

SELECTED ARTICLES

IN VITRO STUDIES OF THE BASIS FOR SULFANILAMIDE THERAPY IN BOVINE MASTITIS

By J. C. Kakavas, Ph.D., Newark, Delaware

(With two charts)

The subject of sulfanilamide therapy in bovine mastitis has received considerable attention in recent years. Although the early reports show contradictory results, more recent controlled studies have revealed that sulfanilamide administered orally to a cow is of little or no curative value in permanently eradicating the streptococci from the udders of cows suffering from acute or chronic mastitis. A new approach to the problem of sulfanilamide therapy in bovine mastitis has been made in this laboratory, and the results of the findings have been reported.[1] It was shown that where sulfanilamide is introduced in large amounts directly into the udder *via* the teat duct, mastitis streptococci are destroyed in most cases. The purpose of this report is to present a study of some of the fundamental principles which govern the activity of sulfanilamide in respect to the infecting organism.

Cultures and mediums

The strains of *Streptococcus agalactiae* employed in this study, with the exception of three cultures, had been isolated in this laboratory from chronic cases of mastitis. Cultures 090R, V-8 and K-151A were supplied by the Lederle Laboratories and represent, respectively, types I, II, and III of Lancefield group B. The identity of the cultures as *Str. agalactiae* was established by means of physiologic tests and serologic reactions, using the precipitin method of Lancefield and the rapid agglutination test. Some of the cultures had been carried on stock culture medium for some time previous to this study, while others were of recent isolation. The test organisms were transferred every twenty-four hours for two to three days to insure rapid growth. Each culture was then diluted in sterile water blanks sufficiently so that 1 c.c. contained approximately 500 to 2,000 living organisms. As will be shown later in this paper, the number of organisms present in the inoculum influences the degree and rapidity of their destruction by the sulfanilamide.

The mediums employed in these studies were tryptose broth and tryptose agar. Their composition was basically the same as described in a previous publication.[1]

Presented before the Section on Sanitary Science and Food Hygiene at the seventy-ninth annual meeting of the American Veterinary Medical Association, Chicago, Aug. 24-27, 1942.

The author is associate bacteriologist, Haskell Research, University of Delaware, Newark.

Effect of temperature on bactericidal activity of Sulfanilamide.—White and Parker[2] demonstrated that sulfanilamide at 20 mg. per cent is germicidal against group A streptococci at 40 C., but not at 37 C. Heishman and Miller[3] also reported that an increase in incubation temperature enhances the bactericidal action of sulfanilamide against group B streptococci. In view of the above reports, the following experiments were conducted to ascertain by quantitative determinations the effect of sulfanilamide on *Streptococcus agalactiae* at different temperatures. The tests were conducted as follows: 20 c.c. of tryptose broth containing 20 mg. per cent of sulfanilamide were placed in 50 c.c. flasks. The control flasks contained tryptose broth only. The test organisms were grown in tryptose broth for twenty-four hours and then diluted so that 1 c.c. contained approximately 500 to 2,000 viable organisms. One c.c. of the diluted culture was placed in each flask. All tests were run in duplicate; one set was incubated at 37 C. and the companion set was incubated at 40.5 C. At intervals of from five to twelve hours, a small portion was withdrawn from each flask and placed in serial dilutions to determine the number of living organisms at each interval. With some of the cultures the experiment was repeated with a variation in the inoculum which consisted of over one million organisms.

In chart 1, the results on four cultures are presented. The ordinates represent the number of living organisms at different intervals plotted in logarithmic scale, and the abscissas represent the time intervals in hours. The results indicate that the organisms in the flasks which were incubated at 37 C. grew at approximately the same rate in both the control and the sulfanilamide flasks. At 40.5° C. the rate of growth was first retarded in both flasks, but soon a divergence occurred whereby the organisms in the control flask continued to grow until the maximum growth was reached, which was approximately the same as that in the 37° C. flasks; however, the organisms in the flasks with the 20 mg. per cent sulfanilamide were rapidly decreasing in number until all the organisms were killed.

There was some variation in the number of hours it took to sterilize the various cultures. In some cases, the organisms were killed in less than twenty hours, whereas in others, it took slightly over thirty hours. In chart 2, the results of the tests in which the inoculum contained more than 1,000,000 bacteria

TABLE 1

Bactericidal properties of sulfanilamide on Streptococcus agalactiae determined by the cup-plate test

Hrs. incubation of Petri dish with sulfanilamide before subculturing	STREPTOCOCCUS AGALACTIAE CULTURE																							
	1A				1B				1C				2A				3B				3C			
	No. bacteria per plate				No. bacteria per plate				No. bacteria per plate				No. bacteria per plate				No. bacteria per plate				No. bacteria per plate			
	438		4380		141		1410		400		4000		80		800		251		2510		80		800	
	a*	b**	a	b	a	b	a	b	a	b	a	b	a	b	a	b	a	b	a	b	a	b	a	b
24	—	+	+	+	—	+	+	+	—	+	+	+	—	+	—	+	+	+	+	+	—	+	+	+
48	—	+	—	+	—	+	+	+	—	+	+	+	—	+	—	+	—	+	+	+	—	+	—	+
72	—	+	—	+	—	+	+	+	—	+	+	+	—	+	—	+	—	+	+	+	—	+	—	+
96	—	+	—	+	—	+	+	+	—	+	+	+	—	+	—	+	—	+	+	+	—	+	—	+
120	—	+	—	+	—	+	+	+	—	+	+	+	—	+	—	+	—	+	+	+	—	+	—	+
Inhibition Zone	20 mm.		18 mm.		12 mm.		6 mm.		15 mm.		8 mm.		15 mm.		10 mm.		15 mm.		5 mm.		20 mm.		15 mm.	

Hrs. incubation of Petri dish with sulfanilamide before subculturing	4A				4B				090R				V-8				K-151A				F-12a-4			
	No. bacteria per plate				No. bacteria per plate				No. bacteria per plate				No. bacteria per plate				No. bacteria per plate				No. bacteria per plate			
	575		5750		430		4300		560		5600		144		1440		106		1060		348		3480	
	a	b	a	b	a	b	a	b	a	b	a	b	a	b	a	b	a	b	a	b	a	b	a	b
24	—	+	—	+	—	+	+	+	—	+	—	+	—	+	—	+	—	+	+	+	—	+	—	+
48	—	+	—	+	—	+	—	+	—	+	—	+	—	+	—	+	—	+	—	+	—	+	—	+
72	—	+	—	+	—	+	—	+	—	+	—	+	—	+	—	+	—	+	—	+	—	+	—	+
96	—	+	—	+	—	+	—	+	—	+	—	+	—	+	—	+	—	+	—	+	—	+	—	+
120	—	+	—	+	—	+	—	+	—	+	—	+	—	+	—	+	—	+	—	+	—	+	—	+
Inhibition Zone	20 mm.		15 mm.		18 mm.		12 mm.		28 mm.		25 mm.		20 mm.		20 mm.		25 mm.		18 mm.		25 mm.		16 mm.	

*a = Resutslts of subcultures made from Cup-Plate at 10 mm. from edge of cup.
**b = Results of subcultures made from Cup-Plate at 30 mm. from edge of cup.

+ = Viable organisms present.
— = No viable organisms.

are shown. Although the final results were comparable to those in which the inoculum was small, the bactericidal effect of sulfanilamide in 20 mg. per cent at 40.5° C. varied directly to the amount of inoculum. The results of these tests demonstrate that sulfanilamide in concentration of 20 mg. per cent is bactericidal against Lancefield group B streptococci only when the incubation temperature is elevated above 40° C. In this respect, Lancefield group B streptococci are affected in the same way by sulfanilamide as the streptococci of Lancefield group A. These results further explain the failure of sulfanilamide to destroy *Str. agalactiae* infection of the udder when the drug is administered orally. Since streptococci mastitis is a nonfebrile infection, and since the limit of tolerance for the cow for sulfanilamide administered orally is a level of 20 mg. per cent in the blood and milk, it is not possible to destroy the streptococci in the udder.

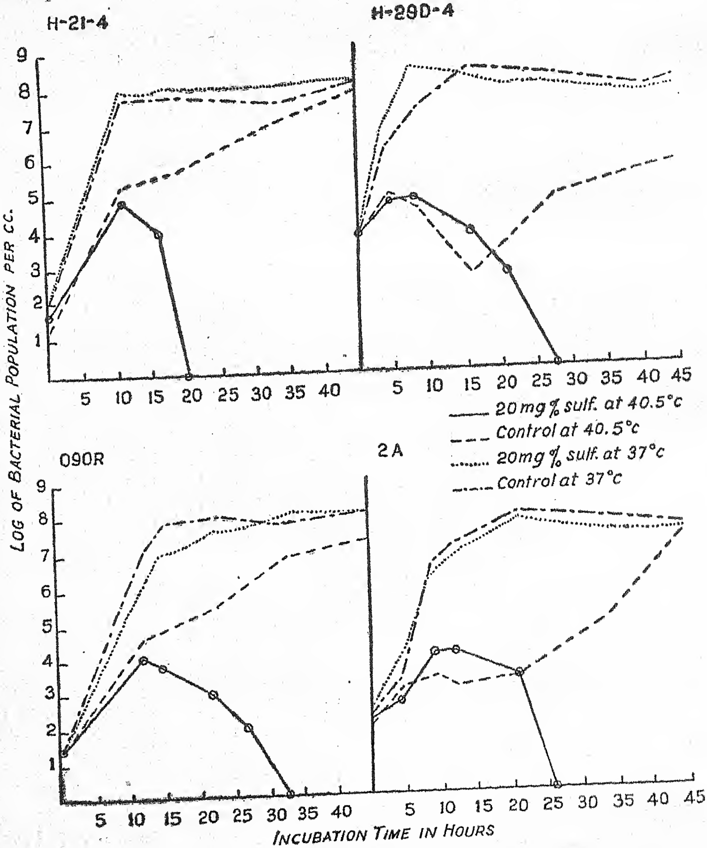

Chart 1. Effect of Sulfanilamide on *Streptococcus agalactiae* in vitro tests

Agar cup-plate method.—To simulate conditions comparable to those of the cow's udder when sulfanilamide is introduced directly into the lactiferous sinus, the following tests were conducted with the agar cup-plate method. Each culture of *Str. agalactiae* used in these tests was run in duplicate. In one Petri dish, the number of organisms in the inoculum was ten times that of the companion Petri dish. One milliliter of the diluted culture was placed in each Petri dish and 30 c.c. of tryptose agar were

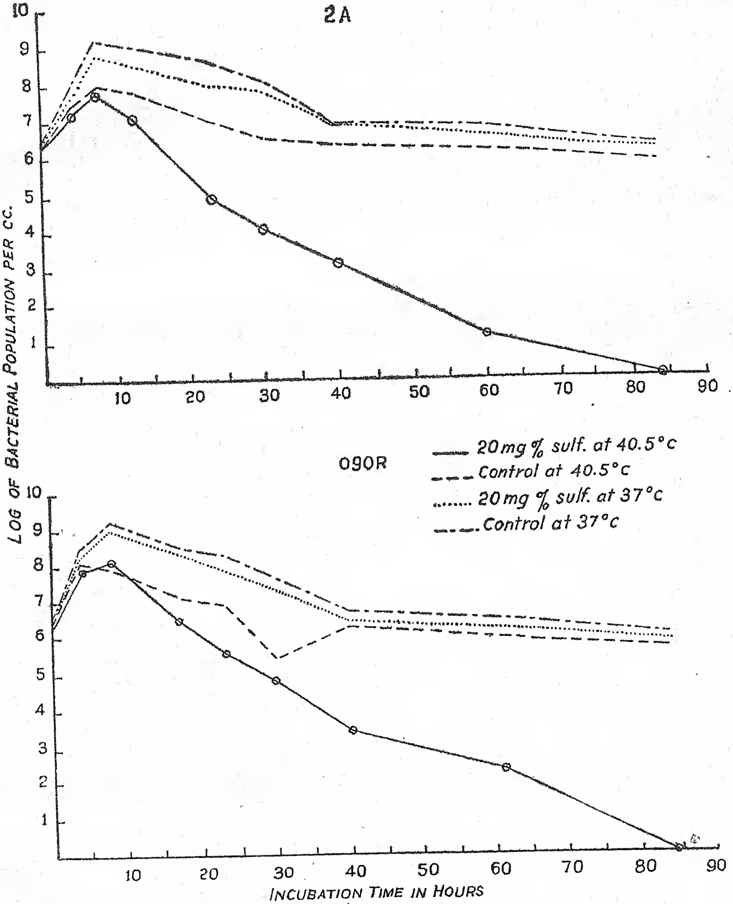

CHART 2. Effect of sulfanilamide on *Streptococcus agalactiae*—in vitro tests using large inoculum

poured on and thoroughly mixed with the culture. A sterile marble was placed in the centre of the agar. When the agar hardened, the marble was removed, and the cup formed by the marble was filled with approximately 1 c.c. of 37·8 per cent homogenized suspension of sulfanilamide in liquid petrolatum. The preparation of the homogenized sulfanilamide has been described in a previous publication.[1] The Petri dishes were then placed in the incubator at 37·5° C. and at intervals of twenty-four hours, subcultures were made from the plates by cutting small pieces of agar at approximately 10 mm. and 30 mm. distance from the edge of the cup and inoculating into tryptose broth. The subcultures were then incubated at 37·5° C. and the presence or absence of growth was recorded at the end of seventy-two hours. The Petri dishes were incubated for 120 hours, and at the end of this period, the zone of inhibition was measured from the edge of the cup.

TABLE 2

Effect of inoculum on the size of inhibition zone by sulfanilamide on Streptococcus agalactiae (F-12a-4) —cup-plate test

Hrs. incubation before sub-culturing	No. bacteria per Petri dish							
	348		3480		34800		348000	
	a	b	a	b	a	b	a	b
11 . . .	—	+	—	+	+	+	+	+
26 . . .	—	+	—	+	—	+	+	+
46 . . .	—	+	—	+	—	+	+	+
70 . . .	—	+	—	+	—	+	+	+
94 . . .	—	+	—	+	—	+	+	+
125 . . .	—	+	—	+	—	+	+	+
142 . . .	—	+	—	+	—	+	+	+
Inhibition Zone	25 mm.		16 mm.		12 mm.		8 mm.	

*a = Results of subcultures made from cup-plate at 10 mm. from edge of cup.
**b = Results of subcultures made from cup-plate at 30 mm. from edge of cup.
+ = Viable organisms present.
— = No viable organisms.

The amount of sulfanilamide which diffused through the agar was determined at the end of the 120-hour period at two points; namely, approximately 10 mm. and 30 mm. from the edge of the cup. The sulfanilamide determinations were made by cutting small pieces of agar and placing them in test tubes which were immersed in boiling water to melt the agar. This was then diluted with distilled hot water sufficiently high so that when the test was completed, a reading could be made with the standard sulfanilamide tubes. The test for sulfanilamide was made according to the method of Marshall and Litchfield.[4] The sulfanilamide concentration in the agar at the end of the test period was, on the average, about 730 mg. per cent at 10 mm. distance and 400 mg. per cent at 30 mm. distance.

TABLE 3

The inhibitory effect of sulfanilamide by streptococcus agalactiae (F-12a-4) metabolites determined by the cup-plate test

Petri dish No.	Hours incubated before adding sulfanilamide	Subculture growth before adding sulfanilamide	Subculture growth after adding sulfanilamide	Size of inhibition zone
1	0	+	—	15 mm.
2	3	+	—	8 mm.
3	8	+	+	0
4	19	+	+	0
5	33	+	+	0
6	Control	+	+	0

Number Bacteria per plate = 1620.

The results of this study are summarized in Table 1. It will be noted that sulfanilamide was bactericidal against all the strains studied, as shown by the inhibition zone around each cup. It should be noted, too, however, that the size of the zones varies with the different strains. The smallest inhibition zone was that of strain 1B with a 6 mm. radius, and the largest inhibition zone was that of strain 090R with a 25 mm. radius. It should be further noted that the Petri dishes containing the smaller inoculum developed larger zones of inhibition compared to those with larger inoculum. This is particularly well demonstrated in Table 2.

In an attempt to find an explanation for this variation in inhibition as a result of the difference in the size of the inhibition zones, the following cup-plate experiment was conducted. A series of Petri dishes were prepared in the same manner described previously, using strain F-12a-4 as the test organism. The plates were incubated at 37·5° C. and the sulfanilamide-oil preparation was added at different intervals in the agar cup. In Table 3, the result of this test shows that when the organisms had an opportunity to grow for a period of approximately eight hours before sulfanilamide was added in the cup, no inhibition zone developed around the cup. It would appear, therefore, that when the organisms are allowed to multiply without the interference of sulfanilamide during the early growth stages, bacterial metabolites accumulate in the medium which nullify the bactericidal effect of sulfanilamide. The difference in susceptibility of the various strains to sulfanilamide may be explained on the basis of the above findings; namely, that group B streptococci produce a sulfanilamide inhibiting substance or substances and that some strains produce more active or more abundant inhibiting metabolites than others.

P-aminobenzoic acid and sulfanilamide activity for group B streptococci.—The problem of the nature

Table 4

Antisulfanilamide activity of p-aminobenzoic acid for Streptococcus agalactiae in tryptose broth

	Tube number												
	1	2	3	4	5	6	7	8	9	10	11	12	13
Sulfanilamide-mg. %	1,000	1,000	1,000	1,000	1,000	1,000	1,000	1,000	1,000	1,000	1,000	0	0
P-aminobenzoic acid-gm.	0·001	0·002	0·003	0·004	0·005	0·006	0·007	0·008	0·009	0·01	0	0·01	0
F-12a-4 Culture—1 : 100,000	0·1cc.	0·1	0·1	0·1	0·1	0·1	0·1	0·1	0·1	0·1	0·1	0·1	0·1
Growth in original tubes	—	—	—	—	+	+	+	+	+	+	—	+	+

Table 4A

Results of subcultures made from above tubes into tryptose broth

Hours incubated before subculturing	Tube number												
	1	2	3	4	5	6	7	8	9	10	11	12	13
11	—	—	—	—	—	—	+	—	+	—	—	+	+
24	—	—	—	+	+	+	+	+	+	+	—	+	+
48	—	—	+	+	+	+	+	+	+	+	—	+	+
72	—	—	—	—	+	+	+	+	+	+	—	+	+
96	—	—	—	—	+	+	+	+	+	+	—	+	+
123	—	—	—	—	+	+	+	+	+	+	—	+	+

of the bactericidal action of sulfanilamide has been made much clearer by Woods and Fildes[5] who discovered that p-aminobenzoic acid* possesses antisulfanilamide activity. It subsequently was shown by Woods[6] that yeast extracts contain a sulfanilamide inhibiting substance, and he presented evidence to indicate that the yeast factor may be p-aminobenzoic acid. Fildes[7] considered that p-aminobenzoic acid was an essential metabolite for bacteria. Rubbo and Gillespie[8] have claimed the recovery of p-aminobenzoic acid from *Clostridium acetobutylicum* and showed that the acid acts as a growth factor for this organism. Since it may be possible that Lancefield group B streptococci may also produce this acid as an essential metabolite, the following experiment was conducted to determine whether p-aminobenzoic acid exerts the same inhibitory effect on sulfanilamide for group B streptococci as it does for other bacterial species.

A series of 13 tubes were set up, each containing 10 c.c. of tryptose broth. Into each of the 11 tubes, 100 mg. of sulfanilamide was added. P-aminobenzoic acid in increasing amounts was also added, the first tube receiving 0·001 gm., and the tenth, 0·01 gm. Tubes 11, 12, and 13 served as controls for sulfanilamide, p-aminobenzoic acid and tryptose broth, respectively. Strain F-12a-4 was used as the test organism. Approximately 240 bacteria were added in each tube. The tubes were then incubated at 37·5° C., and at 24-hour intervals subcultures were made into tryptose broth, using a 4 mm. loop.

* The p-aminobenzoic acid used in this work was supplied by the E. I. duPont de Nemours and Company.

Results

The data presented in Tables 4 and 4A show that 0·005 gm. of p-aminobenzoic acid was sufficient to nullify the bactericidal effect of 1,000 mg. per cent sulfanilamide. In the sulfanilamide control tube (No. 11), which contained 1,000 mg. per cent of the drug, the organisms were killed within a period of eleven hours or less. The maximum concentration of p-aminobenzoic acid (0·01 gm.) used in this experiment had no inhibitory effect on the organisms. In Table 4A, in which the results of the subcultures are recorded, it will be noted that 0·003 gm. and 0·004 gm. of p-aminobenzoic acid exerted partial inhibition on sulfanilamide activity since the organisms were not killed until after forty-eight hours' incubation. When p-acetylaminobenzoic acid was substituted for the p-aminobenzoic acid, the sulfanilamide activity was not inhibited. These results are shown in Table 5. It is of interest to note that the loss of activity of the acetylated form of the acid corresponds to the loss of the bactericidal activity of the conjugated sulfanilamide. These findings lend further support to the theory that sulfanilamide exerts its bacteriostatic action by competing (on account of similarity in molecular structure) with p-aminobenzoic acid in some enzymatic reaction essential to growth.[7] Further studies in the field of the antisulfanilamide factors and the possible discovery of substances which will neutralize their activity should lead to valuable and far-reaching results.

TABLE 5

Effect of p-acetylaminobenzoic acid on sulfanilamide activity for Streptococcus agalactiae in tryptose broth

%	Tube number 1	2	3	4	5	6	7	8	9	10	11
Sulfanilamide-mg. =	1,000	1,000	1,000	1,000	1,000	1,000	1,000	1,000	1,000	0	0
P- acetylaminobenzoic acid-gm.	0·0001	0·001	0·005	0·008	0·01	0·015	0·02	0·025	0	0·025	0
F - 12a - 4 Culture - 1 : 100,000.	0·1cc.	0·1	0·1	0·1	0·1	0·1	0·1	0·1	0·1	0·1	0·1
Growth in Original Tubes.	—	—	—	—	—	—	—	—	—	+	+

Results of subcultures made from above tubes into tryptose broth

Hours incubated before subculturing	Tube number 1	2	3	4	5	6	7	8	9	10	11
11	—	—	+	—	+	—	—	+	—	+	+
24	—	—	—	—	—	—	+	+	—	+	+
48	—	—	—	—	—	—	—	+	—	+	+
72	—	—	—	—	—	—	—	—	—	+	+
96	—	—	—	—	—	—	—	—	—	+	+
123	—	—	—	—	—	—	—	—	—	+	+

DISCUSSION

Although it is not possible at present to formulate a concise explanation for the mode of action of sulfonamide drugs on microorganisms, certain facts have been revealed which have an important bearing on the use of these drugs in chemotherapy. In the light of the results presented in this study, at least three factors must be taken into consideration in the treatment of *Str. agalactiae* infection of the bovine udder. In the first place, at normal body temperature the teat duct, and the clinical result of those studies have been reported in a previous publication.

In addition to the temperature and the drug concentration, a third factor has been discovered which influences the activity of sulfanilamide. Among the many bacterial (37° C) sulfanilamide temperature (37° C.) sulfanilamide in the concentration of 20 mg. per cent is not germicidal against *Str. agalactiae*. A rise of three to four degrees above the normal temperature is required to destroy these organisms with 20 mg. per cent sulfanilamide. Since streptococci mastitis is usually a nonfebrile infection, and since it is not possible to raise the sulfanilamide level in the body of the cow much above 20 mg. per cent without producing serious damage to the animal, the oral administration of sulfanilamide is of little or no therapeutic value in the treatment of streptococci mastitis.

In the second place, evidence is presented to show that sulfanilamide is germicidal for *Str. agalactiae* at 37° C. in concentrations above 100 mg. per cent.* By analogy it may be assumed that since a high level of sulfanilamide concentration is undoubtedly established in the mucosa of the stomach and small intestine when the drug is given orally, similar levels may be attained in the mammary tissue if the drug is given *via* the teat duct, and the clinical results of those studies have been reported in a previous publication.

In addition to the temperature and the drug concentration, a third factor has been discovered which influences the activity of sulfanilamide. Among the many bacterial metabolites, p-aminobenzoic acid, which is claimed to be an essential growth-substance for bacteria and which Ansbacher[9] claims to be a vitamin of the B complex group, has been found to be a sulfanilamide inhibiting substance. From the experimental data presented in this report, *Str. agalactiae* produces metabolites which counteract the bactericidal effect of sulfanilamide. No attempt was made to identify the nature of this inhibiting substance. However, p-aminobenzoic acid was found to nullify sulfanilamide activity against organisms of the Lancefield group B in the same manner as that manifested for the other bacterial species that have been reported.

* *In vitro* experiments conducted in this laboratory have shown that considerable variation exists in susceptibility to sulfanilamide by these organisms; some strains were killed with 100 mg. per cent; others required 800 mg. per cent.

SUMMARY

(1) The results of these *in vitro* tests reveal that at body temperature (37° C.), sulfanilamide in a concentration of 20 mg. per cent has no bactericidal effect against Lancefield group B streptococci. However, a concentration of 20 mg. per cent of sulfanilamide will destroy these streptococci at a temperature of 40·5° C.

(2) In order for sulfanilamide to be bactericidal against group B streptococci at 37·5° C., it is necessary to have a concentration of the drug of over 100 mg. per cent and this high drug level must be attained during the early bacterial growth phase.

(3) P-aminobenzoic acid has been found to counteract the germicidal properties of sulfanilamide on the Lancefield group B streptococci. Under the conditions of these experiments, it was found that one part by weight of p-aminobenzoic acid nullified the germicidal effect of 25 parts by weight of sulfanilamide for strain F-12a-4.

(4) It appears from the results of these experiments that the therapeutic basis of sulfanilamide for Lancefield group B mastitis streptococci is based on at least three factors: the temperature of the animal at the time of treatment; the concentration of the drug at the focus of infection; and the sulfanilamide-inhibiting substances which may be present in the infected area.

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ABSTRACTS

The water economy of farm animals. I. Leitch and J. S. Thomson (1944). *Nutrition Abstracts and Reviews* 14 (2), 197-223

Although water metabolism is an important physiological factor having considerable bearing on animal production, the subject has not received the attention it deserves from research workers. In the published literature, most of the reference is concerned with cattle, references being very few on other farm stock.

Since the dry matter of a ration is generally adjusted to the metabolic needs of the consumer, the existence of a relationship of total water intake to the dry matter of feed might be considered probable. The available data, however, show the lack of such straightforward relationship. The absence naturally suggests several ascertainable causes which may influence water intake.

A close examination of several methods of disposal of ingested water should furnish data on which the requirement can be based. The disposal of water from the body takes place through faeces and urine. A portion is vaporized to dissipate heat increment incident to food consumption. In growing or fattening animal, water is retained for the newly-built tissues. In milch cows, water is disposed of in the milk.

The water content of the faeces is independent of the protein content in the ration and of plane of nutrition. When, however, the ration includes a roughage of lower digestibility, the faeces voided is more moist. Although data are few to study the possible correlation between the moisture and other constituents of faces, it is noted that moisture content increases with increasing crude fibre and nitrogen-free extract, but by no semblance of mathematical proportion. The fæcal moisture in cattle can be approximately reckoned as 80 per cent in steers and dry cows, and 85 per cent for milch animals. In percentage the water content in the faeces of cows in milk seems only slightly higher than that of dry animals, but as the total weight of faeces voided by the former is much greater, the difference becomes highly significant from the standpoint of water requirement. The quantity of urine in cattle is determined primarily by the amount of some non-nitrogenous substance or substances derived from roughages. If the protein ingestion is high, the urinary volume goes above that determined by the roughage intake. In steers and dry cows on maintenance ration or roughage, the weight of urine excreted will be of the order 5—7 kg. daily. From the existing data it is difficult to explain how the environmental temperature should affect the urinary volume.

Water lost as vapour from skin and lungs can be determined directly in the calorimeter or indirectly by determining the 'insensible loss of weight' minus the weight of methane and the balance of carbon dioxide expired over oxygen taken in. By using both methods, data have been collected which on critical examination show that the loss of water by vaporization depends on: (1) Condition of coat; a heavy coat of hair reduces heat emission by radiation and conduction and thus an extra burden is put on water evaporation, (2) Plane of nutrition; with the rise in plane of nutrition, the amount of heat to be dissipated increases which in turn necessitates greater loss of water by vaporization, (3) External temperature; the observations indicate that water losst by

vaporization has a minimum of 2 kg. at 5° C. or 6°C. and increases slowly to about 2·5-4 kg. at 15°C., with a rapid increase to as much as 7 kg. at 21°C. and a slower further increase to an unknown limit; these observations have, however, been considerably influenced by the variable plane of nutrition and coat cover of the subjects; (4) Atmospheric humidity; although theoretically high humidity would be expected to depress vapourization, the limited number of observations made suggested that humidity has little effect.

When water lost by vaporization is plotted against total heat emission the curve obtained is roughly S-shaped, which is predictable also on theoretical grounds.

Pulse and respiration rates are intimately connected with the burden of heat disposal by radiation and evaporation. Records of pulse and respiration counts in several breeds of cows in relation to milk production and summer and winter temperatures show that both rates are capable of great change. The data when properly interpreted indicate that both pulse and respiration rates are higher in breeds of higher average milk yields; that high respiration rate is not always associated with higher milk yield, while higher pulse rate always is. Acceleration of pulse rate allows dissipation of heat both by radiation and evaporation whereas acceleration of respiration helps heat emission by evaporation only. Hence in high milk secretion, association of low respiration with high pulse rate would be advantageous in effecting economy of water for milk production. Under tropical condition at 36°C., the average respiration rate per minute of Holstein, cross-bred and zebu are 107·0, 89·3 and 46·0 respectively. The deterioraton of European cattle in the tropics is obviously due to the failure of heat disposal by radiation and the shift of the burden on to water evaporation, resulting not only in the decline in milk yield but also in the breakdown of constitution. Between Holstein and zebu, although disposal of heat varies in mode there appears to be no difference in the capacity of heat emission. But such is not the case between show and ordinary dairy cows. In a show cow producing 3,000 gallons of milk in a lactation, the heat emission by evaporation entails vaporization of 30-40 kg. of water which is almost double the amount of an ordinary dairy cow. This large evaporation seems to suggest important adaptation in respect of pulse and respiration rates, such as high pulse and low respiration count. Whatever be the nature of the adaptation, it is only the type of cow, which is capable of evaporating large amounts of water, that is likely to maintain high production in the tropics. Whether this would be true in practice or not, only further experimentation can reveal. So far as ordinary dairy cows are concerned, close shearing and the provision of shade are the only practical expedients which can help in maintaining their milk production in the tropics.

Since skin happens to be a medium from which heat emission takes place by evaporation and radiation, the factors which affect skin temperature have important significance. Skin temperature rises with the rising plane of nutrition. It is doubtful whether this is an advantage. In fattening steers, heat emission and skin temperature seem to go in the reversed direction with the degree of utilization of absorbed food. As exposure to sun raises the skin temperature above that of the body this should adversely affect the normal mode of heat disposal.

When the outgo of water through various channels enumerated above is reckoned against the intake of both performed and potential water in feeds, a satisfactory balance can be obtained. A proper knowledge of various channels of outgo under varied dietetic and environmental conditions should thus help in evaluating the water requirement.

The water consumption of sheep, like that of the cow, varies with the type of ration, plane of nutrition and external temperature. Pregnancy seems to impose higher water requirements. Grazing on fresh pastures may cut the water requirement to nil. On the average, the requirement is 2 litres water per kg. dry food consumed.

The recorded data on water requirement of the horse are meagre. By one estimate, it is 2-3 litres of water per kg. dry food consumed. The other estimate gives 35-45 litres daily.

For the growing pig, a water to dry feed ratio of 3 : 1 is considered satisfactory; at bacon weight, however, the ratio is narrowed. The consumption of water by farrowing sows is slightly greater at higher external temperature. The water requirement of pregnant and lactating sows has been calculated to be 8-12 lb. and 40-50 lb. per day respectively.

The water consumption of poultry varies with live weight, external temperature and egg-production. In Leghorn hens in lay, the water requirement has been calculated at 250 gm. daily. A few observations on water loss in vaporization have been made on fowls, which seem to agree generally with those recorded on cattle. [S. C. R.]

The semen of animals and its use for artificial insemination. JAMES ANDERSON (1945). *Technical Communication* Imperial Bureau of Animal Breeding and Genetics, Edinburgh

ARTIFICIAL insemination has aroused wide interest in recent years on account of its enormous possibilities in the development and improvement of livestock production. Since the publication by the Imperial Bureau of Animal Breeding and Genetics, in 1933, of a review on artificial insemination by Dr Arthur Walton, a voluminous literature has accumulated on

the various aspects of the subject and there has been a great demand for a comprehensive and up-to-date resumé of information and results so far obtained. The present communication by Dr Anderson has not only removed this long-felt want but has also supplied a timely stimulus to workers on the subject, especially at the moment when the methods of artificial insemination may have to be applied extensively in improving and replenishing the livestock population of the world so severely depleted by the war.

Literature up to 1943 has been reviewed in the main text of the publication, which is divided into three parts, and later information has been embodied in a supplement. Part I reviews exhaustively literature dealing with the study of various semen characteristics, such as volume, motility, concentration of sperms, pH, decline of motility of stored semen, morphology, effect of exercise on semen, fertility and the interrelationship between various semen characteristics of different farm animals, the study of factors affecting semen production, physico-chemical properties of semen, physiology of spermatozoa, problems of storage and transport of semen and the examination of semen. The quality of semen, so far as its fitness for artificial insemination is concerned, can be roughly estimated by determining the concentration and motility of the sperm, but the precise evaluation would require a detailed examination of the semen with regard to appearance, volume, motility, concentration, pH and pH change on incubation, proportion of abnormal spermatozoa, respiration rate, resistance and longevity of sperms and freedom of semen from bacterial contamination. In part II is embodied a lively discussion about the uses and advantages of artificial insemination, application of the method in different countries, its limitations and objections, and the factors affecting the insemination results. Artificial insemination has the outstanding advantage over natural mating in that it provides a means for increased use of sires, for the evaluation of young sires, for prolonging the period of use of valuable sires, for overcoming difficulties due to size difference in males and females, for the maintenance of satisfactory conception rate, for increased use of sires in monogamous species, for hybridization, for controlling genital diseases and for economic service particularly to small herds. While discussing the problem of utilization of artificial insemination methods for the genetical improvement of stock, the author has emphasized the importance of providing suitable environment. He stresses that the successful utilization of the method would demand energetic action on the part of the government concerned for a systematic programme of work on a practical and scientific basis by setting up throughout the country artificial insemination centres adequately equipped and staffed with specially trained technicians. Part III describes in detail the actual technique of semen collection, the different types of apparatus used for different species of animals, handling and examination of semen, preparation of the semen for insemination, process of insemination and the storage of semen. Collection of semen may be made with the help of an artificial vagina or in some species by massaging the accessory genital organs or by electrical stimulation in the rectum and the lumbar region. The insemination is effected by injecting with a special syringe a small quantity of diluted semen into the cervix of the female or by depositing the semen at the bifurcation of the uterine horns. Introduction of gelatinized sperm capsules into the vagina or the cervical canal of the female has also been adopted lately as a successful method of insemination. A small chapter is included in part III on the management of sires for breeding. Part III is particularly useful to artificial insemination workers, as it gives most minute details about practical aspects of the subject.

The publication comprises 150 pages of printed matter with a large bibliography and is illustrated with 19 plates and diagrams which should be extremely useful to practical workers. A copy of this publication should be an invaluable reference book for every artificial insemination worker in English-speaking countries. [P. B.]

Die Entwicklung des Riemser Adsorbatimpfstoffes gegen Maul-und Klauenseuche und seine Herstelung (The development of Riems foot-and-mouth disease adsorbate vaccine and its preparation). O. WALDMANN, G. PYL., K. O., HOBOHM, and H. MOHLMANN (1941). *Zbl. Bakt. I (Orig.)* 148, 1-15

THE adsorbate vaccines are characterized by the active principle (toxin, anatoxin, virus) being attached to the surface of a finely divided substance, thereby suffering a peculiar change in its antigenic action. The authors review the use of adsorption in enzymology, diphtheria immunization and the field of virus diseases.

The results so far obtained in studying the effect of adsorption on viruses, with particular reference to their antigenic properties, are summarized as follows :

1. Adsorption has no effect on the virus. Example : Equine encephalomyelitis (must be accepted with reserve as it is based on meagre work). 2. Non-infectious or weakly infectious virus sets up good immunity as adsorbate. Example : fowl-pest (Nieschulz strain). 3. Fully virulent virus loses its infectivity through adsorption without losing its immunizing power. Examples : poliomyelitis and fowl leucosis. These results are however based on laboratory experiments and have not been tried out extensively in practice. The only adsorbate vaccine against a virus disease which has been so tried out is the one used in the control of foot-and-mouth disease.

Earlier work on vaccination against foot-and-mouth disease, though extensive, was quite unsuccessful. The use of formolized virus by French workers, however, showed that the prospect was not altogether hopeless. This vaccine was intensively studies by workers in various countries and in Germany it was found that the vaccine could protect 100 per cent of guinea-pigs and 66 per cent of cattle against generalization of the infection. This method was, however, unsuitable for field application as a considerable proportion of batches of the vaccine were infective. The optimum concentration of formol lay within such a narrow range that on one side the vaccine was often infective and on the other it failed to protect; this optimum could not be constantly attained. Recently, some Danish workers studied the adsorption of this virus on aluminium hydroxide and found that, under certain conditions, the adsorption was so strong that the virus could not generally be eluted by chemical means; and, by subcutaneous inoculation in guinea-pigs no disease, but immunity, was set up, the degree of which corresponded with the quantity of adsorbate injected. On intracutaneous inoculation into the foot-pads, however, the adsorbate was still infective but this infectivity could be removed by heating at 25°-37°C. without loss of immunizing property. When used in cattle such a vaccine set up the disease instead of immunizing and the results could have no practical application.

Such was the state of affairs when work on the problem was begun at Riems (The German Foot-and-mouth Disease Research Station is situated at the Baltic island of Riems, off the coast of Greifswald). Earlier Work confirmed the findings of Danish workers and a constantly safe and useful vaccine for cattle could not be made even by more prolonged incubation. This difficulty was overcome by the addition of 0·05 per cent formalin and 48-hour incubation at 25°C. which sufficed to make a constantly safe and efficient vaccine. This work showed that a solution of this difficult problem had been provided by the action of formalin on the virus stabilized by adsorption. The vaccine has been used on over three million animals in the field; none of them developed the disease. Some of the inoculated animals when infected 10-14 days afterwards proved immune to massive infection.

The method of preparation of the vaccine is described in detail under the following headings. The interested reader should refer to the original: (1)Obtaining the virus including choice of production strains and method of infecting the virus-producer animals; (2) Preparation of the virus containing material for incorporation in the vaccine; (3) Mixing, drawing off and incubation of the vaccine; (4) Chemical test of the various reagents required and the vaccine itself; and (5) Biological test of the vaccine for safety, bacterial contamination and immunizing capacity. [M. A.]

Fertility and hatchability when the environmental temperature of chickens is high. BURT W. HEYWANG (1944). *Poultry Science* **23**, 334.

THE influence of high environmental temperature on the fertility and hatchability of eggs laid by White Leghorn pullets has been investigated. The birds were kept at normal, rather than at artificially controlled, temperature conditions. They were trapnested and the eggs, collected from the nests at hourly intervals, were kept in a refrigerator at 55°F. till incubated. They were saved for incubation on the six week days and placed in the incubator the following Monday, during 12 periods between March 29 and August 21, 1943. During this entire period the same White Leghorn cockerels remained with the different groups of pullets.

The data on fertility and hatchability were grouped into six lots depending upon whether the eggs were saved during a period when the maximum temperature was between 80°-84·9°, 85°-89·9°, 90°-94·9° 95°-99·9°, 100°-104·9° or 105°-109·9°F. The average for each of the ranges was determined from the individual figures of the period.

The figures for eggs laid by 108 pullets, when examined, showed that when the average maximum environmental temperature was 82·8°, 86·2°, 93·0°, 97·5° and 101·8°F., the fertility of their eggs was, respectively, 91·9, 93·6, 91·8, 88·8 and 82·9 per cent, and the percentage of chicks hatched from their fertile eggs was, respectively, 78·9, 77·7, 78·4, 71·8 and 68·0.

The eggs laid by 63 pullets of the above group, when the average maximum air temperature was 106·8°F., were also incubated. The average figures of fertility and hatchability for this set of birds, when the average maximum environmental temperature was 82·8°, 86·2°, 93·0°, 97·5°, 101·8° and 106·8°F., were, respectively, 92·5, 92·4, 91·6, 86·8, 80·4 and 73·8 per cent for fertility, and 78·8, 79·3, 78·5, 72·2, 67·9 and 55·1 per cent for hatchability from fertile eggs.

The experimental results were analysed statistically and $\bar{p}$ was determined for the fertility and hatchability of the eggs laid at different environmental temperatures. This showed that at the average maximum temperatures of 101·8° and 106·8°F. fertility and hatchability were significantly lower than those of the eggs laid at the average maximum temperatures of 82·8°, 86·2° and 93·0°F.

It is therefore concluded that fertility and hatchability are lowered when chickens are kept under high environmental temperatures. [T.S.K.]

Studies in bovine mastitis—Modes of spread of *Streptococcus agalactiae* infection in dairy herds. A report on observations organized by

the Agricultural Research Council of the United Kingdom. *Imperial Bureau of Animal Health*, **May 1944, Review Series No. 2**

THIS report deals with the researches that were carried out in 20 dairy herds consisting of various breeds of cows, in five different centres extending from Ayrshire to Kent over a period of 21 months. The important cause of bovine mastitis which reduces the milk yield of dairy herds is *Streptococcus agalactiae* though other streptococci, staphylococci and diphtheroid bacilli cause mastitis in cattle. Mastitis due to staphylococci is more frequent and important than was once supposed. The observations described in this report indicate clearly the routes by which infection occurs and the cause of persistent herd infection.

The methods of collection, sampling and examination of both milk and teat swabs that were adopted as a standard throughout the investigation have been described in detail. These examinations have shown that *Str. agalactiae* is a common inhabitant of the bovine udder or teat canal, even in the absence of clinical symptoms. Various workers have recorded the incidence of mastitis due to *Str. agalactiae*. Minett reported its isolation from milk of 38·3 per cent of cows the infection varying from 10 per cent to 71 per cent in different herds. But there is no recorded information regarding the occurrence of this organism on the exterior of the teat. In this investigation of 16,482 samples of milk examined *Str. agalactiae* was isolated from 38·8 per cent. The results of examination of milk and teat swabs of different herds are given in tabular form. It was noticed that some cows which gave consistently positive milk tests gave very irregular results from teat swabs, while in others, where milk tests were negative, positive teat-swab tests were obtained. The relation between positive milk samples and positive teat swabs are given. Of the 2177 positive milk samples 816 or 37·5 per cent were positive for teat swabs. On the other hand of the 3599 negative milk samples only 526 or 14·6 per cent were positive for teat swabs. But of the total positive teat swabs, 61·5 per cent were associated with positive milk samples. It was proved that the successive positive teat swabs may not be due to true skin infection but to repeated contamination by milkers' hands. Sores, cracks and other blemishes on the teats become infected very frequently with *Str. agalactiae*. It was also noted that heavy skin infection may also occur in the absence of any lesions on the teats. It was also proved that *Str. agalactiae* infection often persists in the dry udder and also on the teats during the dry period. The persistent infection in dry cow is a factor in the maintenance of infection in the herd.

A search for *Str. agalactiae* in sites other than the cow's udder was made with the following findings :

1. The occurrence of *Str. agalactiae* on the teats of heifers that have not been milked is extemely rare. But *Str. agalactiae* frequently appears in the milk of first-calf heifers during their initial lactation.

2. *Str. agalactiae* could not be isolated from the throat and nostrils of calves or from the vagina of cows. Some workers, have claimed isolation of this organism from the mouths of calves suckling infected cows about eight hours after suckling.

3. *Str. agalactiae* can be isolated from the great majority of the hands of milkers who are daily handling the teats of the cattle in any average herd.

4. *Str. agalactiae* is not a natural human parasite. When found on the milkers' hands or in the throat, this is simply the result of contamination from milk.

5. *Str. agalactiae* has been isolated from churn handles, lids, door, knobs, broom handles and from the cups of milking machines.

6. The air of cattle sheds is contaminated with *Str. agalactiae*, but air-borne infection is of minor importance as compared with contact infection.

A study of the relation between *Str. agalactiae* in milk and clinical symptoms indicated that 283 or 14·3 per cent of 1,977 samples of milk yielding *Str. agalactiae* were associated with signs of clinical mastitis, while only 129, or 3·7 per cent of 3,460 samples of milk not yielding *Str. agalactiae* were associated with signs of clinical mastitis. In other words, signs of clinical mastitis were approximately four times as frequent when *Str. agalatiae* was present in the milk as when it was absent.

Methods of control are detection of infected cases, eliminating them from the herd, or isolating and milking the infected cows last. [M.K.S.]

The effect of feeding cottonseed meal as the only concentrate on several properties of milk. 1. Fat, total solids and ash content. P. G. MILLER and G. H. WISE (1944). *J. Dairy Sci.* **27**, 275-279

THE object of the experiment was to determine the effect of feeding cottonseed meal as the only concentrate on several properties of milk to a group of Holstein herd. This was compared with a control group of an equal number of similar animals which was fed on a concentrate mixture consisting of four parts of corn gluten meal, two of wheat bran, two of ground corn and two of ground oats. The roughage allowed to both the groups was the same and comprised either corn or soybean silage or pasture. In addition, both the groups were given an allowance of 2 per cent bone meal and 1 per cent salt. The experimental observation extended to a period of 16 consecutive months.

The data presented showed that four months after the animals were placed on their respective feed, the milk yielded by the experimental group had comparatively a lower percentage of total solids, fat and

solids-not-fat than that of the control group. There was a decrease in the ash content also which, however, followed later. [T.A.]

Toxaemie jaundice in sheep. *Australian Agricultural Newsletter, Release No. AGN/104, October, 1945, Canberra*

It is expected that results of observations and experiments begun during the past 12 months will point the way to satisfactory methods of control of toxaemic jaundice in sheep.

The investigation is being conducted by the Toxaemic Jaundice Committee, representing the Council for Scientific and Industrial Research, the Departments of Agriculture of Victoria and New South Wales, and the Melbourne University Veterinary Research Institute. It is financed jointly by the Australia Wool Board and the Australian Meat Board.

Results at the experimental farm suggest that proteins of high biological value, such as casein, have a protective action against the damage to the liver associated with toxaemic jaundice. It is also suggested that the low intake of protein during late summer and autum contributes to the liver damage, associated with storage of high amounts of copp to be found in the area in which the disease occurs.

Grazing on heliotrope (*Heliotropium europaeum*) increased the death rate in the flock by almo 300 per cent, and deaths with signs of jaundic 21 fold. Strain due to shortage of food contr buted to the deaths in the flock from all cause It would appear that heliotrope causes such adver effects because it favours a higher intake of copp by the sheep than a grass pasture. Surveys of so and pasture are being determined. Results, it expected, will suggest possible means of preventi intake of copper by the sheep, or of preventing exce storage of copper in the liver.

There is evidence to suggest that the assimilatio and storage of copper by sheep is regulated by th mineral in the soil and in the plant. Molybdenu is one mineral element which can reduce coppe storage in the grazing animal. It occurs in widel varying concentrations in plants. When present i high concentration in the plant it may cause coppe deficiency in the sheep although the copper con centration in the plant remains normal. It seem possible that, if molybdenum is in very low concen tration in the plant, a very high storage of coppe may be built up by the grazing sheep.

THE MAYNARD GANGA RAM PRIZE.

Applications are invited for the award of the Maynard Ganga Ram Prize of Rs. 3,000 for a discovery or an invention or a new practical method which will tend to increase argicultural production in the Punjab on a paying basis. The prize is open to all, irrespective of caste, creed or nationality and Government servants are also eligible for it. Essays and theses are not accepted. The prize will be awarded for something practically achieved as a result of work done after the prize was founded in 1925. Competitors in their applications must give a clear account of the history of their invention or discovery and mu produce clear evidence that it is the result their own work. In the case of an improved cro details of parentage, evolution and history and botanical description are necessary.

The Managing Committee reserves to itself th the right of withholding or postponing the prize no satisfactory achievement is reported to it, or t reduce the amount of the prize or to divide it the quality of the entries justifies this decision.

Entries should reach the Director of Agricultur Punjab, Lahore, not later than 31st October 194

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